

## ALPHA-1-ANTITRYPSIN DEFICIENCY AND PULMONARY EMPHYSEMA: THE ROLE OF PROTEOLYTIC ENZYMES AND THEIR INHIBITORS

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MUCH of the emphasis of present-day research into the aetiology of pulmonary emphysema stems from the discovery by Laurell and Eriksson (1963) of an abnormality in the electrophoretic protein pattern of a number of sera undergoing routine examination. The subjects concerned were found to have a marked deficiency of  $\alpha_1$ -antitrypsin ( $\alpha_1$ -at), the main component of the  $\alpha_1$ -globulin fraction. Further enquiry revealed a high incidence of pulmonary emphysema among those with this abnormality, which appeared to be transmitted by an autosomal recessive gene (Eriksson 1964, 1965).

The antitryptic activity of human serum was first recognized many years ago (Camus & Gley 1897), but there had been no suggestion before the paper of Laurell and Eriksson in 1963 that its absence was linked with pulmonary disease. Since then a large number of cases of emphysema associated with  $\alpha_1$ -at deficiency have been described, and it is now clear that they form a distinct clinical group with early onset of irreversible exertional dyspnoea and a strong tendency for the lower zones of the lungs to be the most severely affected. Kueppers and Bearn (1966a) have suggested that in the absence of  $\alpha_1$ -at the release of proteolytic enzymes from leucocytes may damage the lung, and that the predominant destruction of the lower zones may be related to the greater blood flow in that area. In this respect there is a striking contrast with the location of emphysematous lesions in patients with normal  $\alpha_1$ -at, where the upper zones are the most severely affected in over half the cases (Hutchison et al. 1972). Apart from this, the similarities between the two groups of patients suggest that the biochemical and pathological processes which are responsible for emphysema have much in common, whether  $\alpha_1$ -at is present in normal quantities or not. In this review it was therefore not thought reasonable to consider solely that evidence which had been accumulated in relation to  $\alpha_1$ -at deficiency alone, and the discussion extends, where appropriate, to cover the more general topic of the pathogenesis of emphysema as a whole.

The release of proteolytic enzymes from the lysosomes of polymorphs and alveolar macrophages is almost certainly of paramount importance, and it has been recognized for very many years (Opie 1905) that the white cells, in fulfilling their defensive role, may at the same time damage the organism. The

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function of the enzyme inhibitors, of which  $\alpha_1$ -at is possibly the most important, is to protect the tissues against such damage. Emphysema can in fact be produced experimentally by the administration of white cell lysosomal extracts as an aerosol, and it seems likely that the release of these enzymes in man is very often due to cigarette-smoking. Emphysema is most uncommon in non-smokers, and in  $\alpha_1$ -at deficiency the available evidence suggests that smoking hastens the onset of the disease.

The very interesting preponderance of males with emphysema in both  $\alpha_1$ -at deficient and non-deficient groups of patients is a topic which so far has received little attention, and evidence derived from research into gout, where a similar male preponderance is found, suggests that oestrogens may prevent rupture of the lysosomal membrane and the consequent release of tissue-damaging enzymes.

There has been considerable argument whether the heterozygotes for  $\alpha_1$ -at deficiency are also more likely to develop emphysema. The author remains unconvinced that they have such a predisposition. The solution of this problem clearly has important practical implications; if such an association were proved it might be necessary to establish a screening programme to detect these individuals. Since they form 10–15% of the population this would be an arduous and expensive undertaking.

Further important information on the nature of this disorder came from an unexpected source with the discovery by Sharp et al. (1969) of an association between  $\alpha_1$ -at deficiency and a severe form of neonatal hepatitis. Pathological studies in these infants and in adults suggests that in patients with the deficiency  $\alpha_1$ -at is manufactured in the liver in normal quantities, but in a form which cannot be discharged from the liver cells in the normal way.

### Clinical Features

Eriksson (1964) described the clinical features in the first recorded cases of emphysema associated with  $\alpha_1$ -at deficiency, and subsequently expanded upon his findings in more detail (Eriksson 1965). The main symptom is severe and progressive shortness of breath which presents at an early age, commonly between 30 and 45 years. Since the original description many similar cases have been reported (Briscoe et al. 1966; Guenter et al. 1968; Hunter et al. 1968; Talamo et al. 1968; Tarkoff et al. 1968; Hepper et al. 1969; Welch et al. 1969; Jones & Thomas 1971; Hutchison et al. 1971). The clinical manifestations are very similar to those of emphysematous patients with normal serum  $\alpha_1$ -at, though symptoms generally make their appearance at a much earlier age in those with the deficiency (Jones & Thomas 1971; Hutchison et al. 1972).

Chronic bronchitis is a not uncommon feature, and Eriksson (1965) noted that over half his patients had recurrent attacks of cough with sputum production. Similar findings have been reported by other authors (Tarkoff et al. 1968; Welch et al. 1969), though Talamo et al. (1968) found little evidence of bronchitis in their series. Hutchison et al. (1972) found that about two-thirds of the patients with  $\alpha_1$ -at deficiency had chronic bronchitis as defined by the Medical Research Council (1965) and that there was a very similar proportion among

emphysematous patients with normal serum  $\alpha_1$ -at. In both groups there were patients in whom the features of emphysema preceded chronic bronchitis and vice versa; some patients had never produced sputum at any time, and others had simply complained of one or more attacks of acute bronchitis. The presence of chronic bronchitis made no significant difference to the common respiratory function tests in either group; chronic bronchitis, in short, appears to have little to do with the onset of emphysema, nor is there any evidence that it influences the course of the disease whether  $\alpha_1$ -at deficiency is present or not. Both chronic bronchitis (Higgins 1959) and emphysema (Fletcher et al. 1964) are, however, each associated with a third factor, namely cigarette-smoking.

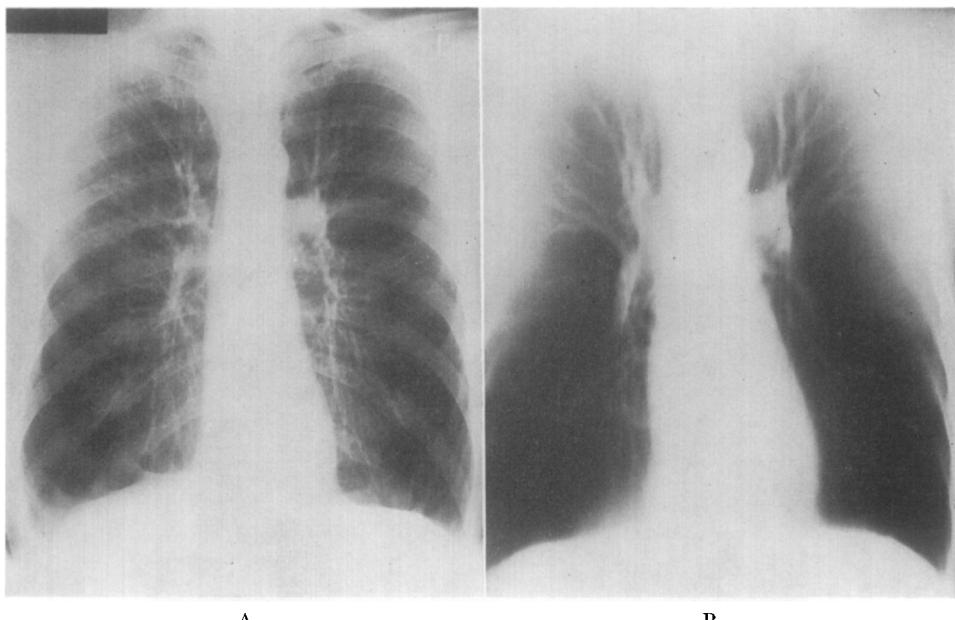


FIG. 1. A, Postero-anterior chest radiograph in full inspiration. B, Whole lung tomogram (13 cm cut). The patient was a male aged 45 with  $\alpha_1$ -at deficiency and severe emphysema. The lungs are overexpanded, with vascular attenuation at both bases. (Reproduced by kind permission of the Editor of the *British Medical Journal*)

The radiological distribution of the lesions in this condition is striking (Figs 1, 2); the lower zones are almost always the most severely affected (Eriksson 1965; Briscoe et al. 1966; Guenter et al. 1968; Hutchison et al. 1971) and this distribution has been confirmed by lung scanning (Welch et al. 1969). In pathological studies, likewise, the main lesions are at the bases and generally have the features of panlobular emphysema (Eriksson 1965; Schleusener et al. 1968; Tarkoff et al. 1968). In emphysematous subjects with normal  $\alpha_1$ -at, however, the lesions are found predominantly in the upper zones in over half the cases (Hutchison et al. 1972), a difference for which no generally acceptable explanation has yet been produced.

Tests of respiratory function reveal abnormalities which are characteristic of emphysema rather than of  $\alpha_1$ -at deficiency as such (Eriksson 1965; Guenter et al.

1968; Hunter et al. 1968; Tarkoff et al. 1968; Welch et al. 1969; Hutchison et al. 1972). In patients with symptoms there is usually severe reduction in the one second forced expiratory volume and in the carbon monoxide transfer factor; the total lung capacity may be much enlarged. The vital capacity, however, may remain within the normal range until a relatively late stage. The arterial CO<sub>2</sub> tension is generally normal or moderately reduced, becoming raised only in the later stages of the disease. A substantial reduction in the arterial O<sub>2</sub> tension is likewise a fairly late finding. The loss of pulmonary elastic recoil reported by Hunter et al. (1968) and by Hepper et al. (1969) would tend to allow collapse of the airways and limitation of flow during expiration.

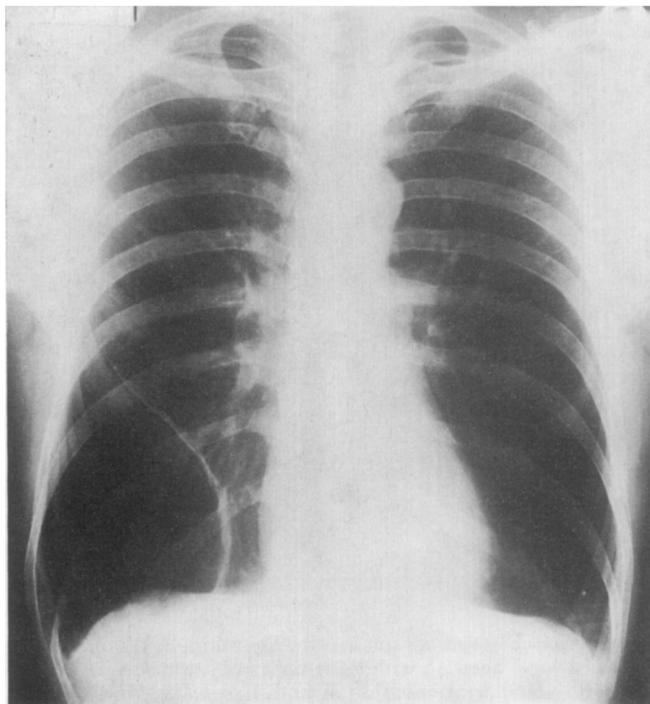


FIG. 2. Postero-anterior chest radiograph in full inspiration. The patient was a male aged 50 with  $\alpha_1$ -AT deficiency and emphysema. There is a large bulla in the right lower zone and vascular attenuation at the left base.

### Prevalence

There are wide variations in the quoted figures for the prevalence of  $\alpha_1$ -AT deficiency among patients with emphysema; in an early study, Kueppers et al. (1964) found only one such subject among 99 patients attending an emphysema clinic, and later Kueppers et al. (1969) identified the condition in 5 of 103 patients classified as having chronic obstructive pulmonary disease. Lieberman (1969) obtained a figure of 10·4% among 66 patients with emphysema. These differences must arise from factors such as the selection criteria (which in some studies were not precisely defined), the population served by the particular unit

and the policy or policies of the physicians referring patients to it. A good case can be made for standardizing the diagnostic criteria, and the appearance of peripheral vascular attenuation or bullae on the plain chest radiograph or on tomograms (Laws & Heard 1962) would ensure that any patients selected do in fact have emphysema, as opposed to other forms of chronic airflow obstruction; admittedly some of the milder cases are bound to be excluded. On this basis, Hutchison et al. (1971) found that 13% of their emphysematous patients had  $\alpha_1$ -AT deficiency, and in a study restricted to severe cases (Hutchison et al. 1972) the figure was no less than 29%; the unit concerned, however, is one in which a special interest is taken in the surgical treatment of emphysema (Pride et al. 1970) and one might, therefore, expect a bias in favour of patients in whom the disease appeared relatively localized, particularly where actual bullae were present. Bullae occur with equal frequency among deficient and non-deficient patients, so that any marked bias from this source seems improbable. The early death of some of those with the deficiency, on the other hand, may even reduce the estimated prevalence. In the present circumstances, therefore, a comparison of the relative frequencies obtained by different units or clinics appears of limited value, and there are also considerable differences in gene frequency between ethnic groups (Kellerman & Walter 1970). There seems no doubt, however, about the high incidence to be found in younger patients; nearly all of a narrowly defined group in whom severe lower zone emphysema has developed between the ages of 30 and 45 are likely to have  $\alpha_1$ -AT deficiency (Hepper et al. 1969; Hutchison et al. 1971). In the author's view we are observing a well defined clinical entity, and the association of  $\alpha_1$ -AT deficiency and pulmonary emphysema seems most unlikely to be due to chance alone.

### Cigarette-smoking

Not all subjects with  $\alpha_1$ -AT deficiency go on to develop pulmonary emphysema, but it is difficult to obtain an estimate of the proportion who do so. Eriksson (1965), in a survey of a Swedish community of about 7000 persons, found 4 subjects with  $\alpha_1$ -AT deficiency, of whom 2 had evidence of emphysema. These numbers are clearly too small for an accurate conclusion to be drawn, but a certain amount of information can be obtained by studying the sibs of  $\alpha_1$ -AT deficient patients who have presented with emphysema; with the small families found in this country today, however, workers in the average chest unit may find it hard to accumulate sufficient data. Nonetheless, there is evidence suggesting that the onset of emphysema may be precipitated by cigarette-smoking. The patients of Briscoe et al. (1966), Hunter et al. (1968), Tarkoff et al. (1968), Hepper et al. (1969) and Hutchison et al. (1971) were all smokers. Some deficient non-smokers have not developed the disease until later in life (Guenter et al. 1968; Jones & Thomas 1971) and some remain symptom-free, with virtually normal pulmonary function, and may have escaped the disease altogether (Hutchison et al. 1971). The total cigarette consumption of deficient patients with emphysema is very much less than that found in patients with a similar degree of emphysema (Hutchison et al. 1972), suggesting that the

deficient subjects may be particularly sensitive to cigarette smoke. Not all the evidence points in this direction however; only half the patients studied by Eriksson (1965) were smokers and Talamo et al. (1968) reported 2 non-smokers who developed symptoms before the age of 35. Some further evidence on the relationship of cigarette-smoking to emphysema will be presented later in this review.

### Serological Methods

Some of the serological methods in common use for the estimation of  $\alpha_1$ -at will now be outlined; their applications will be amplified in subsequent sections.

#### 1. Serum trypsin inhibitory capacity (TIC)

The enzymatic determination of TIC is one of the most commonly employed methods for the quantitative determination of  $\alpha_1$ -at, and has been found to correlate well with radial immunodiffusion. The effectiveness of serum in blocking the action of trypsin on a suitable synthetic substrate can be measured by spectrophotometry. There is, however, considerable variation in the specific activity of commercial trypsin preparations, and a strong recommendation for the use of agreed standards was made in a recent symposium (Mittman 1972).

Similar methods can be used for the measurement of serum elastase inhibitory capacity which is closely related to the TIC.

#### 2. Quantitative determination of serum $\alpha_1$ -at

*Electrophoresis in cellulose acetate* estimates the total  $\alpha_1$ -globulin; it is of limited accuracy but can be used as a screening procedure for the detection of homozygotes for  $\alpha_1$ -at deficiency.

*Radial immunodiffusion* (Mancini et al. 1965) is widely used. Antigen is allowed to diffuse from a central well into agar containing antibody, the area of the precipitate under standard conditions being proportional to the concentration of antigen. The normal concentration of  $\alpha_1$ -at by this method lies between 200 and 250 mg/100 ml.

#### 3. Genetic typing

*Acid starch gel electrophoresis* (Fagerhol & Braend 1965) (Figs 3, 4) allows the  $\alpha_1$ -globulin region to be examined with much greater discrimination. A single variant such as M or Z, although in all probability a homogeneous substance, produces a number of separate bands on the gel due to interference with the reagents in use. Two main bands are seen for each variant, though other fainter bands may also appear. This complicates the interpretation somewhat, but each variant has a specific pattern from which it can usually be recognized; an exception is the MZ phenotype where the Z bands are extremely faint, and crossed antigen-antibody electrophoresis is required for certain identification.

*Crossed antigen-antibody electrophoresis* (Kueppers & Bearn 1966b) (Fig. 5) is performed in two stages; after initial starch gel electrophoresis, a second electrophoresis is applied at right angles into agarose gel containing antibody to  $\alpha_1$ -at.

After staining a number of precipitation peaks become visible from which the particular variant can be identified.

### The Pi System

The early investigations of Eriksson (1964, 1965), based to a large extent on the serum trypsin inhibitory capacity (TIC), suggested that  $\alpha_1$ -at deficiency was transmitted in simple Mendelian fashion by an autosomal recessive gene. With this mode of inheritance, two alternative genes (or 'alleles') may exist at a given chromosome locus; only those homozygous for the deficiency gene would have low levels of  $\alpha_1$ -at and a tendency to develop emphysema; heterozygotes, on the other hand, would not have such low levels of  $\alpha_1$ -at and the disease would not be expressed. In practice, on the basis of the TIC estimation three separate groups

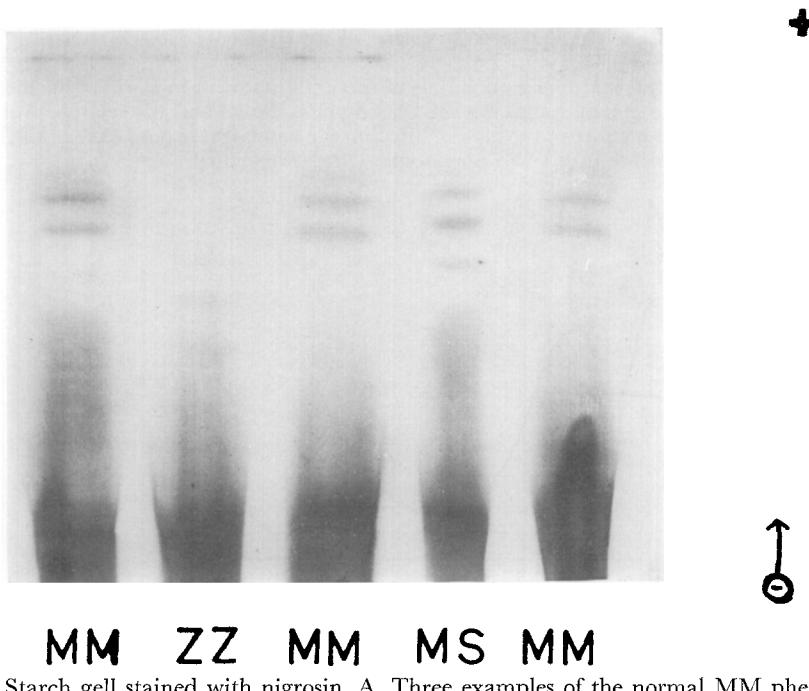


FIG. 3. Starch gel stained with nigrosin. A, Three examples of the normal MM phenotype. B, The ZZ phenotype typical of  $\alpha_1$ -at deficiency. No M bands are visible and faint Z bands of very slow migration rate are seen. C, The MS phenotype. (Reproduced by kind permission of the Editor of the British Medical Journal)

can be identified—normal, 'intermediate' (60% of normal) and 'low' (10%)—corresponding respectively to normal subjects, to heterozygotes and to homozygotes for  $\alpha_1$ -at deficiency (Eriksson 1965; Talamo et al. 1968). Usually the homozygous deficient subjects can be readily distinguished by this method, but owing to the considerable overlap in values, the heterozygotes cannot be separated with certainty from the normal subjects. The term heterozygote is, in any case, frequently employed in an ambiguous sense, as will be seen.

Further investigation has shown that the biochemical abnormalities found in this disorder are complex. The introduction of acid starch gel electrophoresis

(Fagerhol & Braend 1965) allowed the  $\alpha_1$ -globulin region to be examined with much greater discrimination, and by this means it has been shown that  $\alpha_1$ -at may exist in a number of different biochemical forms or 'variants'. A number of

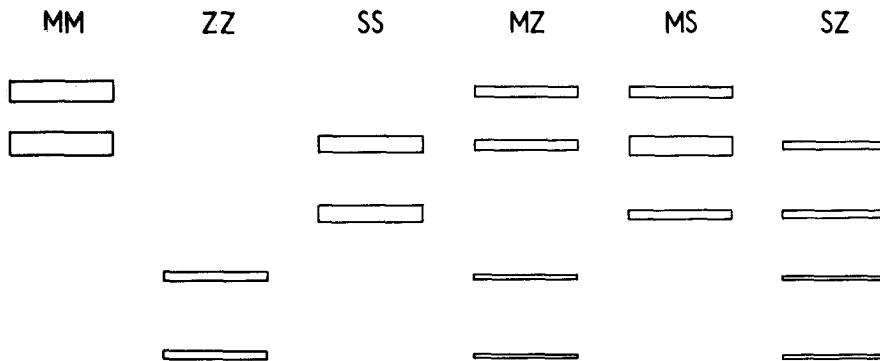


FIG. 4. A schematic diagram of starch gel electrophoretic patterns in 6 different phenotypes. The relationship of the 2 main bands in each phenotype is shown and the relative protein content is indicated by the breadth of the band. Note that the bands from one of each of the M and S pairs coincide in the MS phenotype. (After Fagerhol & Laurell 1970)

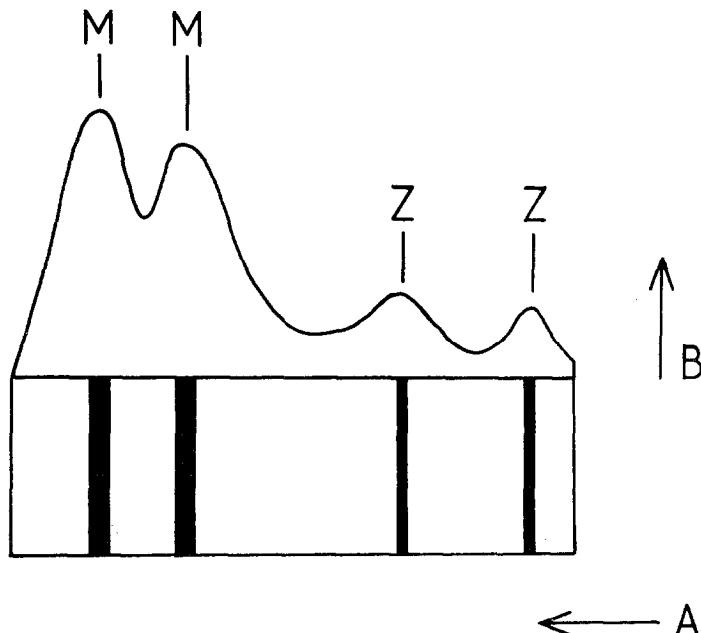


FIG. 5. Crossed antigen-antibody electrophoresis; the precipitation peaks shown in the MZ phenotype. Stage 1: Acid starch gel electrophoresis in direction A, resulting in separation of protein fractions as in Fig. 3. Stage 2: the starch gel strip is removed and placed in an agarose gel plate containing  $\alpha_1$ -at specific antiserum. Electrophoresis is then applied in direction B

such variants have now been described (Fagerhol & Laurell 1970) and are known collectively as the 'Pi' system, the abbreviation standing for protease inhibitor. Each variant has been assigned an alphabetical letter indicating its electrophoretic mobility in acid starch gel. The common type is called M and

2 of the earlier variants to be recognized were termed F and S (fast and slow) as compared with M (for medium). A subject homozygous for the M variant has the 'genotype' MM, the majority of the population falling into this category. Each genotype gives rise to specific biochemical variants (the 'phenotype') and may also be expressed as clinical abnormalities. The variant with the slowest electrophoretic mobility is termed type Z; homozygotes for this variant, who have the genotype ZZ, are the only subjects who have a definite proven tendency towards the development of emphysema. The term ' $\alpha_1$ -at deficiency' is commonly used to refer to this group alone (as in this review), but it is often used by authors to indicate any individual in whom the serum  $\alpha_1$ -at concentration is less than normal (see Table 1). Other uncommon variants, such as I, P, X, V and W, have been described and a large number of possible combinations are therefore possible (Fagerhol & Laurell 1970).

TABLE I. ALPHA-I-ANTITRYPsin CONCENTRATION ASSOCIATED WITH VARIOUS GENOTYPES

| Genotype | $\alpha_1$ -at concentration<br>(mg/100 ml; mean $\pm$ SD) | Percentage<br>contribution<br>(MM = 100%) |
|----------|--|---|
| MM       | 286 $\pm$ 73   | 100                                       |
| ZZ       | 45 $\pm$ 8   | 16  |
| SS       | 149 $\pm$ 23   | 52  |
| MZ       | 164 $\pm$ 44   | 57  |
| MS       | 215 $\pm$ 47   | 75  |
| SZ       | 106 $\pm$ 34   | 37  |

After Lieberman, Gaidulis et al. (1972).

The autosomal recessive concept is therefore a rather simplified version of the truth, though for many practical purposes it is a convenient approximation. The variants in fact behave as fully penetrant co-dominant alleles; that is to say, each of the 2 variants (even type Z) contributes a characteristic 'dose' of  $\alpha_1$ -at to the total (Fagerhol 1969; Lieberman, Gaidulis et al. 1972). To illustrate this point, if the contribution of the common MM genotype is represented as 100%, then the ZZ phenotype is found to contribute on average about 15% and the MZ heterozygote about 57% (50% from M and 7% from Z). The concentrations of  $\alpha_1$ -at contributed by the more important of the individual types are shown in Table 1, and it will be noted that there is considerable variation about the mean values. Only S and Z of the types so far described are associated with significantly low values; occasional cases of emphysema have been observed in association with type SZ (Fagerhol 1972; Cook 1973) but not so far with type SS. The controversy whether heterozygotes (mainly of type MZ and MS) are likely to develop emphysema will be discussed in a separate section.

The differences in electrophoretic mobility of the components of the Pi system are presumably due to differences in molecular structure, though few data on this point are available as  $\alpha_1$ -at is difficult to obtain in a pure form. Fagerhol and Laurell (1970) suggest that the variants are brought about by simple amino acid substitutions. This type of inherited molecular variation (or

'polymorphism') is found in many other systems such as haptoglobin and transferrin, though in most instances there are no clinical effects.

The question arises whether emphysema in the ZZ type is brought about simply by the low plasma level or whether the Z variant is itself less effective as a protease inhibitor. Inspection of the data of Lieberman, Gaidulis et al. (1972) suggests that the activity of the Z variant is the same per mole as that of the other variants. Individuals of type SZ ( $\alpha_1$ -at level 37% of normal) do not apparently have the same risk of developing emphysema as the ZZ subjects and it is possible that a crucial plasma level may lie at 20–30% of normal. What may be more important, however, is the fact that the type Z protein cannot be released from the liver in response to stress as we will see in a later section of this review; there is little information on the response to stress in subjects of type SS, but in 3 cases who underwent liver biopsy (Gordon et al. 1972), there was no evidence of  $\alpha_1$ -at accumulation in the liver as seen in subjects of type ZZ. One of the main defences against emphysema may be not so much the mean basal level as the ability of the liver to discharge  $\alpha_1$ -at and raise plasma level in response to various forms of stress such as infection. Conventional evidence of infection such as chronic bronchitis as defined by the Medical Research Council (1965) may be absent in many cases of emphysema (Hutchison et al. 1972). It is not, however, difficult to imagine that damage may arise at the alveolo-capillary level without associated sputum production.

### Genetics

While the intricacies of Pi typing are of great interest, the only subjects who are definitely proved to be predisposed to emphysema are those of the ZZ genotype. For practical purposes it is therefore quite reasonable to regard the mode of inheritance as conforming to the autosomal recessive pattern. It may be useful at this point to summarize some of the more important consequences:

In a population characterized by random mating without consanguinity, it can be shown (by the 'Hardy-Weinberg' principle) that if  $p$  and  $q$  are the respective gene frequencies for a gene M and for its allele, gene Z (so that  $p + q = 1$ ) then the prevalences of the genotypes MM, MZ and ZZ are in the proportions  $p^2 : 2pq : q^2$ . When the prevalence of the common allele is close to unity, as in the present case, it follows that

$$\text{Prevalence of MZ} = 2 \times \sqrt{(\text{Prevalence of ZZ})}.$$

This simple rule holds good up to a frequency of 1 in 100 for the rare gene.

Eriksson (1965) calculated that the frequency of  $\alpha_1$ -at deficiency in a Swedish population of nearly 7000 was 1 in 1750, giving a heterozygosity rate of about 1 in 20. It follows that the ratio of heterozygotes to deficient homozygotes in the population is about 85 to 1; most affected individuals therefore arise from heterozygous matings, from which (on the classical pattern of recessive inheritance) the ratio of normal to affected offspring is 3 to 1, leaving aside for the present the fact that not all deficient subjects develop emphysema. The mean family size in this country now being less than 3, many of these sibships must

consist entirely of unaffected individuals, as shown in Table 2 (Roberts 1970). Thus about half these families will contain no affected subjects and will escape notice since there is no simple means of detecting the heterozygotes applicable to a large population. Among 8 cases of  $\alpha_1$ -at deficiency (Hutchison et al. 1971), in only one family did the propositus report a suggestive family history. Even when a case does come to light, there is seldom any 'family history' in the conventional sense, and furthermore any attempt to prove that 'non-deficient' cases of emphysema arise through a hereditary abnormality is liable to fail without a specific genetic marker. Before the discovery of  $\alpha_1$ -at deficiency Wimpfheimer and Schneider (1961) postulated that emphysema might be a familial disorder. The present author has studied a number of families with emphysema, normal  $\alpha_1$ -at and a suggestive pedigree. Such pedigrees, however, afford no definite proof unless they are accompanied by a specific biochemical abnormality.

TABLE 2. RELATION BETWEEN FAMILY SIZE AND THE NUMBERS OF AFFECTED INDIVIDUALS

| Size of sibship | Sibships with no affected individuals (%) |
|-----------------|---|
| 1               | 75  |
| 2               | 56  |
| 3               | 42  |
| 4               | 32  |
| 5               | 24  |

After Roberts (1970).

From time to time the question is raised of the possible inheritance of an 'emphysema gene' which acts independently of the  $\alpha_1$ -at deficiency gene. In the first place it must be remembered that there could be no conclusive proof of the inheritance of emphysema without the biochemical marker; secondly, the modern concepts of gene action indicate that every inherited clinical disorder must take its origin from a specific biochemical error, though modifying or precipitating factors may also play their part.

### The Serum Protease Inhibitors

It has been known for very many years that human serum has antitryptic activity (Camus & Gley 1897) and Jacobsson (1955) showed that about 90% of the inhibitory activity was localized to a fraction in the  $\alpha_1$ -globulin region. This fraction subsequently became known as  $\alpha_1$ -antitrypsin but has a wider range of activity than its name implies, the term 'protease inhibitor' now being generally used for this class of protein. Schultze et al. (1962) described some of the characteristics of  $\alpha_1$ -at, estimating its molecular weight at 60 000.

Much of the remainder of the serum's inhibitory activity is due to  $\alpha_2$ -macroglobulin, and both this and  $\alpha_1$ -at are active against a number of proteases, trypsin, chymotrypsin and elastase among others (Heimburger & Haupt 1966; Rimon et al. 1966).  $\alpha_1$ -at is the major serum inhibitor of elastase and both

normal and abnormal sera show a very close correlation between their trypsin and elastase inhibitory properties (Senior et al. 1971), suggesting that these properties may depend on a single enzymatic site. Laurell (1972) has pointed out however that *in vivo*,  $\alpha_2$ -macroglobulin has an affinity for trypsin 6 times greater than  $\alpha_1$ -at, so that although  $\alpha_1$ -at is present in greater quantities the effective inhibitory properties of the two substances may be similar.

Substantial variations may take place in the serum levels of the protease inhibitors, and allowance must be made for these if the results of analysis are to be correctly interpreted. There is no sex difference in serum TIC (Shulman 1952; Eriksson 1965), though Ganrot and Scherstén (1967) reported that  $\alpha_2$ -macroglobulin was 20% higher in females at all ages. The latter authors also found that  $\alpha_2$ -macroglobulin levels were 2–3 times as high in infants as in adults, reaching a peak between 1 and 3 years of age and levelling off by the age of 30. Serum  $\alpha_1$ -at at birth is within the adult normal range (Laurell, 1968).

Of particular importance is the rapid elevation of TIC which takes place in response to many conditions; this was seen by Shulman (1952) in acute infection, in myocardial infarction, after administration of typhoid vaccine and in a variety of neoplastic disorders, especially where there was evidence of tissue destruction.  $\alpha_1$ -at is one of a group of proteins whose serum levels rise in response to stress, as demonstrated after surgery by Crockson et al. (1966).  $\alpha_2$ -Macroglobulin, however, does not appear to behave in this way. The reacting group includes fibrinogen, haptoglobin and caeruloplasmin among others, and they are often known collectively as 'acute phase reactive proteins'.

The reactions to such stress in patients with  $\alpha_1$ -at deficiency are very different. After administration of typhoid vaccine, Kueppers (1968) observed a substantial rise in serum  $\alpha_1$ -at in both normals and heterozygotes with a peak at the third or fourth day, but there was virtually no response in those homozygous for the deficiency. The values in many of the heterozygotes in fact rose into the normal range, making even more difficult the task of identifying the genetic status from the TIC alone.

Considerable alterations in serum proteins occur during pregnancy. Ganrot and Bjerre (1967) observed in normal subjects that serum  $\alpha_1$ -at levels doubled by the end of pregnancy, and both these workers and Horne et al. (1970) found that  $\alpha_2$ -macroglobulin increased by 20–30%. In a subject with  $\alpha_1$ -at deficiency it was shown that TIC rose into the heterozygote range during pregnancy (Sharp et al. 1969) possibly by placental transfer from the fetus, but remained at the deficient level when an oral contraceptive agent was later taken by the same subject. Lieberman et al. (1971) found that oral contraceptives brought about a moderate rise in TIC in most heterozygotes and showed that this effect was brought about by the oestrogenic component; a progesterone analogue produced no change in TIC either in normal subjects or in the heterozygote. These authors suggested that oestrogens might be used as a provocative test for the positive identification of the heterozygous state in subjects with a subnormal TIC. The use of such a procedure on a large scale, however, does not seem justifiable at present, in the absence of conclusive evidence that heterozygotes have an increased susceptibility to pulmonary disease.

### The Heterozygous State

There has been much debate whether the heterozygous subjects are also more liable to develop emphysema. This controversy continues and the contenders seem, if anything, to have drawn further apart. The issue is clearly one of practical as well as theoretical importance; if such an association does exist a case can be made for detecting the heterozygotes before they develop serious disease. We must remember, however, that the heterozygous state occurs in some 10–15% of the population, so that a great deal more evidence is required before the expense of such a screening procedure could be justified.

Differences in methodology may be one cause of this divergence of opinion. The quantitative methods, TIC and radial immunodiffusion, are commonly used but, as previously discussed, individual heterozygotes may not be identified due to the overlap with the normal range. This does not, of course, prevent a statistical comparison of two or more groups of subjects. Although the absolute values may differ from laboratory to laboratory because of lack of agreed standards, the results in any single study would, one hopes, be internally consistent. The qualitative method, crossed antigen-antibody electrophoresis, is thought to be a more certain method of detecting the heterozygous state (particularly important in type MZ), but is in use in relatively few laboratories owing to its technical difficulties. There is also some ambiguity in the use of the term heterozygote. Where quantitative studies reveal subjects with 'intermediate' levels of  $\alpha_1$ -at it is commonly assumed that they are of the MZ genotype. This is very often true, but most MS subjects also will fall into this range. Strictly speaking the term heterozygote refers to any one of the many possible combinations of two unlike alleles; most of these are in fact seldom seen.

A further reason for this controversy may lie in the variation in the modes of selection of patients and other individuals for study. Groups of patients with pulmonary disease, or more specifically with emphysema, naturally form the basis of most studies, though there may be many differences in the selection criteria. Obligatory heterozygotes (parents or children of homozygotes for the deficiency) may also be studied; a disadvantage is that the number of available subjects tends to be small, and in practice only the parents are old enough to have any chance of manifesting the disease. Examination of the heterozygotes in a healthy population represents another possible method of study.

In three studies (Talamo et al. 1968; Jones & Thomas 1971; Hutchison et al. 1972) the mode of patient selection, the methodology and the conclusions appear very similar. Patients with radiological evidence of emphysema were selected and tested by one or both of the quantitative methods (TIC and radial immunodiffusion). In each study, the values obtained in the patients without  $\alpha_1$ -at deficiency were very much greater than those found in obligatory heterozygotes and did not differ significantly from those of the controls; if anything, they were a little higher. The variances also were no different, the results suggesting that, although both groups must contain a proportion of heterozygotes, there is no apparent excess among patients with emphysema.

A rather different approach was employed by Welch et al. (1969) who found

a somewhat higher percentage of subjects with intermediate TIC among chest clinic patients than among normal controls, though the difference was not actually significant ( $P=0.12$ ). There was no consistent pattern of disease moreover, and only 2 of the 17 presumed heterozygotes had any evidence of emphysema, a sharp contrast to the typical radiographic abnormalities seen in the deficient homozygotes. They concluded that there was little evidence of an association between the heterozygous state and emphysema.

A number of authors whose approach to the problem was rather similar to that of Welch et al. (1969) have, however, reached the opposite conclusion. Lieberman (1969) observed a higher incidence (17%) of subjects with intermediate TIC among patients reported to have emphysema than among controls (8.5%), a difference that was suggestive but not actually significant ( $P=0.1$ ). In a larger series of patients, however, Lieberman et al. (1969) found the incidence (18.1%) of heterozygotes to be very significantly greater than that of the controls (4.7%) ( $P<0.001$ ). The diagnostic criteria were not defined in either of the latter two studies, so that a comparison with other investigations is not an easy matter. Similarly, Stevens et al. (1971) studied 11 patients with intermediate deficiency selected from patients of whom a number had been admitted to hospital with pulmonary disease. The older patients (5 in number) had radiological evidence of emphysema together with evidence of airflow obstruction. This study indicates that heterozygotes may indeed develop emphysema, but does not appear to offer any proof that they are more likely to do so than those with normal  $\alpha_1$ -at. Studies in apparently healthy subjects (Mittman et al. 1972) suggest that the incidence of airflow obstruction and of lung scan abnormalities was greater in those with intermediate values of  $\alpha_1$ -at than in normals, but again there is no firm evidence that such subjects have emphysema, or are more likely to develop it in the future.

In occasional studies, the method of crossed antigen-antibody electrophoresis has been used to investigate this problem. Kueppers et al. (1969) observed that 25 of 103 patients classified as having 'chronic obstructive pulmonary disease' were heterozygotes, a figure significantly greater than that found in a control group of healthy subjects (14 of 100). This control figure has seemed unduly high, but it now appears (Kueppers 1971) that subjects of both the MZ and MS types are included. In the latter report rather different results were obtained in a second centre using the same methods; both the incidence among normals (7%) and among the patients (15%) were considerably lower, a difference for which there is no obvious explanation. The diagnostic criteria used by Kueppers et al. (1969) do not include a radiological assessment, and patients with disorders other than emphysema could be included; a comparison of the results with those of other studies is again hardly possible. If subjects of type MS are at special risk, one would surely expect those of the SS or SZ phenotype to develop emphysema in the same way as patients of type ZZ, and to do so with equal frequency. There are, however, only a small number of reported cases of SZ subjects with emphysema (Fagerhol 1972; Cook 1973) and none of type SS to the author's knowledge.

Studies on obligatory heterozygotes do not suggest that they have any

special tendency to develop emphysema. Eriksson (1965) interviewed 64 heterozygous relatives of index cases and could find no evidence of an increased incidence of pulmonary disease, though radiological and physiological investigations were not performed. Hutchison (1973) could obtain no evidence of severe emphysema among parents of  $\alpha_1$ -at deficient patients with the disease. Seven of these were still living, their mean age being 66 (range 52–80); 12 had died at a mean age of 70 (range 55–92) and from the stated causes of death there was no evidence that emphysema was implicated. Guenter et al. (1971) investigated 12 obligatory heterozygotes over the age of 40 and found no excessive incidence of pulmonary disease.

Much of the argument must surely stem from the differences in general design and in patient selection which are found in the various studies reported in the literature. While admitting to a modicum of bias, the reviewer is inclined to believe that the heterozygous state is not an important cause of *severe emphysema*, using this expression to indicate patients with progressive exertional dyspnoea, radiological evidence of pulmonary vascular loss or destruction, severe airflow obstruction and impaired carbon monoxide transfer. This was the conclusion reached in 3 studies which showed some degree of uniformity in their design, but does not by any means rule out the possibility that heterozygotes may have a tendency towards a moderate degree of airflow obstruction, particularly if they are cigarette-smokers. Whatever the truth of the matter, it would be a great advantage if investigators in this field were to agree upon diagnostic criteria for emphysema. Radiological criteria such as those of Laws and Heard (1962) would probably be the most satisfactory and, it is to be hoped that future reports will include clinical, physiological and radiological details of the subjects who have been studied. In addition, the term 'chronic obstructive pulmonary disease' should be recognized to include a number of disorders other than emphysema. One cannot imagine that agreement on this topic will be reached for some time, but to close the debate for the present it is worth making one general observation. In recessively inherited disorders it is unusual for the heterozygotes to manifest the clinical features of the disease as seen in the affected homozygote, though a biochemical abnormality is commonly detectable. This principle is well illustrated in fibrocystic disease, the disastrous effects of which are virtually unknown in the heterozygote.

#### $\alpha_1$ -Antitrypsin and the Liver

A quite unexpected turn of events was the identification by Sharp et al. (1969) of a number of cases of  $\alpha_1$ -at deficiency among a group of infants with neonatal hepatitis, a condition which often leads to early cirrhosis. Estimates of the incidence of  $\alpha_1$ -at deficiency among such cases range from 18% to 40% (Porter et al. 1972; Aagenæs et al. 1972).

The pathological findings (Sharp 1971; Aagenæs et al. 1972) are of great interest; besides the expected evidence of cellular infiltration, bile duct proliferation, cholestasis and fibrosis, globules of an unusual amorphous material were observed within the liver parenchymal cells. On electron microscopy the globules were found to be situated within the rough endoplasmic reticulum, an

intracellular structure thought to be concerned with the formation of the serum proteins. Furthermore, the globules gave a positive staining reaction after application of fluorescein labelled antibody to  $\alpha_1$ -at. Lieberman, Mittman and Gordon (1972) and DeLellis et al. (1972) have demonstrated exactly the same abnormalities in the livers of adult subjects with  $\alpha_1$ -at deficiency, who for the most part had no other evidence of liver disease. Aagenæs et al. (1972) and Lieberman, Mittman and Gordon (1972) observed similar globules in heterozygotes, though in much smaller quantities. It now seems certain that all subjects in whom such abnormalities appeared, possessed the Z variant either in homozygous or heterozygous form, and so far they have not been seen in association with any other variant (Gordon et al. 1972).

There seems no doubt that the material in the globules represents the missing serum  $\alpha_1$ -at. The occurrence of two such very different disorders as neonatal hepatitis and adult pulmonary emphysema in association with the identical biochemical lesion would, however, be a somewhat unusual event. The two disorders have once been observed in a single family (Sharp et al. 1969), though one would perhaps have expected instances of this kind to occur more frequently. We must remember, however, that in the case of affected sibs the events could well be separated by 30–40 years. Neonatal hepatitis of this type in general carries a poor prognosis, though a minority survive (Porter et al. 1972); clearly not all infants with  $\alpha_1$ -at deficiency develop liver disease, and one must presume that in those affected the condition has been induced by some 'trigger' factor, as has been inferred for its pulmonary counterpart. Australia antigen has been suggested as a possible cause, but most authors have been unable to detect it in their patients (Sharp 1971; Aagenæs et al. 1972). An adult with cirrhosis, emphysema and  $\alpha_1$ -at deficiency has recently been reported (Cohen et al. 1973).

It seems most probable that the abnormality in the molecule of the Z variant interferes critically with the intracellular transport mechanism, resulting in an extremely low plasma level and failure to increase the rate of  $\alpha_1$ -at release from the liver in response to stress.

### White Cell Proteases and Inhibitors

#### *Polymorphs*

The presence of proteolytic enzymes or 'leucoproteases' within the leucocytes was recognized many years ago by Opie (1905); he also observed that serum was an effective inhibitor of these enzymes, and even at that early date he saw that the function of the inhibitory factor was to protect the tissues against the powerful enzymes produced by the leucocytes. This concept is today firmly established and it is now clear that the white cells in defending the organism may at the same time be responsible for severe damage to it. Grob (1949) found that the serum inhibitors were mainly in the  $\alpha_1$ - and  $\alpha_2$ -globulin fractions, and Eriksson (1965) suggested that the release of leucocyte proteases could be responsible for the development of pulmonary emphysema in subjects with  $\alpha_1$ -at deficiency. Kueppers and Bearn (1966 a) shortly afterwards confirmed that these enzymes could be inhibited by  $\alpha_1$ -at, though at that time it seemed that the enzymes were

released as the white cells became necrotic during an inflammatory episode. It has already been noted, however, that overt inflammation does not necessarily precede the development of emphysema whether  $\alpha_1\text{-at}$  deficiency is present or not (Hutchison et al. 1972).

In the last few years much has been done to clarify the nature of these enzymes. Janoff and Scherer (1968), during an investigation into modes of vascular injury, obtained evidence that the lysosomal granules of neutrophil leukocytes contain a potent elastase and showed that this enzyme has quite different properties from porcine pancreatic elastase, the enzyme commonly used to test serum inhibitory capacity. Compared with the latter, the lysosomal enzyme is much less susceptible to the action of serum inhibitor, and is more active at pH 7.0. Janoff and Zeligs (1968) observed that the lysosomal extracts could damage the vascular basement membrane, which is of significance in view of the previous demonstration by electron microscopy (Martin & Boatman 1965) that lesions in the pulmonary capillaries are one of the early features of emphysema. Lieberman and Kaneshiro (1972) obtained from the leucocytes of purulent sputum an elastase of similar properties to the lysosomal enzyme and quite probably identical with it. Galdston et al. (1972) suggest that in subjects with  $\alpha_1\text{-at}$  deficiency the outcome is more favourable in those who have low polymorph elastase levels. Yet another enzyme, a collagenase, has been detected in the polymorph granules (Lazarus et al. 1968); this, too, may contribute to tissue damage.

Janoff (1972 a) found the main serum inhibitor of the polymorph lysosomal elastase to be  $\alpha_1\text{-at}$  itself, though Ohlsson (1971) considered that  $\alpha_2\text{-macroglobulin}$  was equally effective. A further inhibitor is found in the non-granular fraction (or 'cytosol') of the neutrophil extract (Janoff & Blondin 1971); the latter inhibitor is, however, present in low concentration and differs from  $\alpha_1\text{-at}$  in that trypsin and pancreatic elastase are unaffected. It is thought to play a limited role in the protection of the white cell itself, but would be relatively ineffective as a protection against any large-scale extracellular release of protease.

#### *Alveolar macrophages*

The alveolar macrophages play a most important part in the defence of the lung against inhaled toxic agents and, as in the case of the circulating polymorphs, it seems that they too may be responsible for damage to the lung itself. An advantage in the study of these cells is that they can be obtained in relative isolation by lavage of the bronchial tree at post mortem, from experimental animals or from living subjects by passage of a catheter into the bronchial tree under local anaesthesia, as for bronchography. Extracts of these cells also contain an elastolytic enzyme (Janoff et al. 1971), though this has only about 10% of the activity of the leucocyte elastase. On the other hand, Rosenberg et al. (1972) have shown that the macrophage elastase is much less readily inhibited by  $\alpha_1\text{-at}$  and  $\alpha_2\text{-macroglobulin}$  than is the polymorph elastase and the authors suggest that it may play a part in the lysis of lung elastin in emphysema, particularly in cases with normal serum  $\alpha_1\text{-at}$ . In the macrophage cytoplasm an inhibi-

tor of the neutrophil elastase has been described (Blondin et al. 1972) and this may act as yet another defence mechanism, but so far as the author is aware, a specific inhibitor of the macrophage elastase has not yet been described.

### Experimental Emphysema

In some of the first experiments of this type, Gross et al. (1964) were able to produce emphysema in rats by intratracheal administration of the enzyme papain. The relevance of this work to human disease was not entirely clear as papain is not found in human serum and has no elastolytic properties, but Kimbel et al. (1972) have now produced emphysema in dogs by exposing them to an aerosol containing the polymorph elastase fraction; emphysema was however produced very much more readily (Janoff 1972 b) by a macrophage extract, and it is of interest that the lesions were found to have a centrilobular distribution. The production of emphysema in animals requires relatively large doses of the active agent, and there is much still to be learnt about the parts played by the various enzymes and their inhibitors in the pathogenesis of emphysema in man, and about the factors which cause enzyme release from polymorphs and macrophages.

### Cigarette-smoking and Emphysema

In an earlier section of this review it was suggested that cigarette-smoking may hasten the onset of emphysema in patients with  $\alpha_1$ -at deficiency. The evidence taken by itself is not conclusive, but when emphysema is considered in a rather more general way there seems little doubt that cigarette-smoking plays an important part in its pathogenesis; indeed, patients who have never smoked are hard to find among those with this disease (Fletcher et al. 1964; Hutchison et al. 1972). In pathological studies, Auerbach et al. (1963) observed that the previous cigarette consumption was related to the degree of emphysema, and Anderson et al. (1970) found no severe cases of emphysema among proved non-smokers.

At this point we must return to the subject of the alveolar macrophages and there is now evidence that cigarette-smoking can produce marked changes in these cells. Pratt et al. (1969) and Harris et al. (1970) obtained alveolar macrophages from healthy subjects by bronchial lavage, and on comparing the properties of the macrophages from smokers and non-smokers quite marked differences were found. Many more macrophages were obtained in each lavage from the smokers, and on electron microscopy important features were the greater size and number of the lysosomal bodies. It seems not unreasonable to assume that in certain circumstances lysosomal proteases may be released from the cell; one of the many toxic substances in tobacco smoke, nitrogen dioxide, has in fact been shown to promote the release of proteases within the lung (Lunan & Freeman 1972).

The metal cadmium may also be a possible toxic agent and chronic industrial cadmium poisoning is known to be associated with emphysema (Smith et al. 1960). Cadmium is also a constituent of cigarette smoke (Nandi et al. 1969) and

in post mortem studies has been found in greater quantities in the livers of patients with chronic bronchitis or emphysema than in those of patients dying from other causes (Lewis et al. 1969; Morgan et al. 1971). This brings the discussion back yet again to the alveolar macrophage; cadmium ions produce a substantial reduction in the oxygen uptake of these cells and the inhibition of a number of important enzyme systems (Mustafa et al. 1971), though it has not so far been shown to cause the actual release of proteolytic enzymes.

We have already mentioned a report (Janoff 1972b) of the experimental production of centrilobular damage by a macrophage lysosomal extract. There is considerable disagreement whether centrilobular emphysema in man can actually produce any important physical disability (Snider et al. 1962; Reid 1967; Mitchell et al. 1970) but from unpublished observations, Martelli et al. (1973) considered that the centrilobular lesion might be responsible for many of the cases of severe upper zone emphysema. Some years ago, Wright and Kleinerman (1963) postulated that centrilobular emphysema might result from the release of enzymes from the macrophages which accumulate in the region of the respiratory bronchioles. Such a hypothesis (rather too conveniently, perhaps) would leave the polymorph proteases as the basis for panlobular emphysema which is generally agreed to be the pathological type found in severe lower zone emphysema, whether the subjects have  $\alpha_1$ -at deficiency or not. Welch et al. (1969) have already suggested that the release of proteolytic enzymes from blood-borne leucocytes in  $\alpha_1$ -at deficiency might account for the predominance of the disease in the lower zones, where the blood flow per unit volume of lung tissue is considerably greater (West 1962); the greater hydrostatic pressure at the lung bases in the upright posture could be a further contributory factor.

The evidence to date then, seems quite strongly in favour of the idea that the various types of emphysema are probably caused by one or more of the constituents of tobacco smoke. A number of heavy smokers never develop emphysema, however, and it therefore seems necessary to postulate that these substances produce emphysema only in subjects with a particular susceptibility (possibly inherited) of which  $\alpha_1$ -at deficiency is one example.

### The Sex Ratio in Emphysema

On the basis of autosomal recessive inheritance, the numbers of males and females homozygous for any biochemical abnormality should exactly balance, though this principle does not necessarily hold good for the clinical manifestations. Eriksson (1965) found in fact that in homozygotes for  $\alpha_1$ -at deficiency over the age of 40 nearly all the males developed emphysema, whereas only half the females did so. Jones and Thomas (1971) and Hutchison et al. (1972) likewise noted many more males than females among their cases. The preponderance of males among emphysematous patients in general is also very marked (Thurlbeck 1963; Burrows et al. 1965; Jones & Thomas 1971; Hutchison et al. 1972), and in the presence of normal serum  $\alpha_1$ -at, emphysema in the female is most unusual before the menopause. The male preponderance is commonly explained either on the grounds that men smoke more than women, that they have a

greater exposure to atmospheric pollutants or that they more readily seek medical advice. Even if true, there is no definite evidence that any of these factors are responsible for the excess of males with emphysema.

It is instructive at this point to turn one's attention away from emphysema for a short time and to contemplate another hereditary disorder in which males predominate, namely, primary gout. In this disorder the ratio of males to females is some 20 to 1 and, again, women do not develop clinical symptoms until after the menopause. Weissmann and Rita (1972) have postulated that tissue damage in acute gout is brought about after phagocytosis of monosodium urate (MSU) crystals by polymorphs. The crystals then come to lie in vacuoles which merge with lysosomes forming so-called secondary lysosomes. The membrane surrounding the latter is normally protected by enzyme inhibitors such as  $\alpha_1$ -at, but in the presence of MSU this protective factor becomes ineffective; MSU is no longer prevented from coming into contact with the lysosomal membrane which then ruptures allowing proteolytic enzymes to be released. Of particular interest is the fact that  $17\beta$ -oestradiol can stabilize the membrane and prevent enzyme release, whereas testosterone has no such effect. These last factors presumably account for the sex distribution in gout, and it seems reasonable to suggest that some similar processes occur in pulmonary emphysema. Lieberman (1971) has in fact reported that both oestrogen and progesterone can prevent experimental papain-induced emphysema, without any increase in the serum levels of protease inhibitor.

### Treatment

There is unfortunately little effective treatment for advanced cases of this disease and as in other cases of severe emphysema clinical management is limited to little more than the administration of antibiotics and oxygen, and efforts to persuade the patient to abandon cigarette-smoking. Lung transplantation is beset by many problems and has not so far improved prognosis.

It is clearly important to detect subjects with  $\alpha_1$ -at deficiency as early as possible and preferably before much lung damage has taken place. Stopping cigarette-smoking is at present the only step likely to improve prognosis. At present, however, subjects generally only come to light after another member of the family has developed serious disease. Replacement of the missing plasma fraction would be desirable, but the half-life of  $\alpha_1$ -at in the body is only some 4 days (Kueppers & Fallat 1969). Makino and Reed (1970) were able to produce a significant increase in serum  $\alpha_1$ -at by plasma infusion in deficient subjects, but this is clearly impracticable in the long run. Liver transplantation has been performed in one patient (Sharp et al. 1969), restoring serum TIC to normal within 2 days, though the patient later succumbed. Future research may produce a long-lasting synthetic substitute for  $\alpha_1$ -at; the development of substances which prevent lysosomal rupture may also have a place in preventing the disease in predisposed subjects. Finally, it is obvious that the necessity for many of these measures would be much reduced if patients stopped smoking cigarettes.

### Summary

The association of  $\alpha_1$ -antitrypsin deficiency ( $\alpha_1$ -at) with pulmonary emphysema is now widely recognized. The main symptom of the condition is progressive shortness of breath, presenting commonly between 30 and 45 years of age. In the advanced case, there is severe expiratory air-flow obstruction, impairment of carbon monoxide transfer and radiological evidence of emphysema predominantly affecting the lower zones. Cigarette-smoking is likely to be an important factor in promoting the onset of emphysema in such subjects.

$\alpha_1$ -at, the main component of the  $\alpha_1$ -globulin fraction, is the major protease inhibitor of human serum,  $\alpha_2$ -macroglobulin being responsible for much of the remaining inhibitory activity. A number of genetically determined variants of  $\alpha_1$ -at can be detected by acid starch gel electrophoresis and are known collectively as the Pi system. The common variant is termed type M, homozygotes having normal serum  $\alpha_1$ -at; homozygotes for type Z, however, have  $\alpha_1$ -at deficiency with a very low serum trypsin inhibitory capacity and have a strong predisposition towards the development of pulmonary emphysema. There has been some controversy whether heterozygotes have a similar tendency, but there is at present no conclusive evidence that they develop emphysema in the severe form often seen in the affected homozygote. Differences in selection criteria appear to be an important cause of many of the disagreements that have arisen.

$\alpha_1$ -at deficiency is also found in a significant proportion of cases of neonatal hepatitis, the occurrence of two syndromes of such different character in association with a single biochemical lesion being a somewhat unusual phenomenon. Similar pathological appearances are, however, observed in the two conditions, accumulations of  $\alpha_1$ -at being demonstrable within the hepatic parenchymal cells; this is thought to be due to failure of the intracellular transport system to handle the abnormal Z variant. In normal subjects there is a substantial increase in the rate of  $\alpha_1$ -at release from the liver after infection and other forms of stress, but in  $\alpha_1$ -at deficiency this presumably protective response does not occur.

Emphysematous patients with normal  $\alpha_1$ -at do not fall into a single homogeneous group, the upper zones bearing the brunt of the disease in over half the cases. In other respects however, the clinical, physiological and radiological features are very similar to those of patients with the deficiency. It is logical to assume therefore, that the pathological processes have much in common, whether  $\alpha_1$ -at deficiency is present or not.

It now appears that the destruction of the lung substance, which is the principal feature of emphysema, is brought about by the excessive release of proteolytic enzymes within the lung. The lysosomal granules of both polymorphs and alveolar macrophages contain potent elastolytic enzymes, and lysosomal extracts can cause emphysema when administered experimentally directly into the trachea or as an aerosol. One of the most important serum inhibitors of the polymorph lysosomal elastase, is  $\alpha_1$ -at itself, with  $\alpha_2$ -macroglobulin possibly playing a lesser part; yet another inhibitor is found in the macrophage cytoplasm. One of the main functions of these inhibitors must be to protect the structural elements of the lung from proteolytic enzymes, though the elastase

derived from the alveolar macrophage is much less readily inactivated by the inhibitors at present known.

Cigarette-smoking is thought to encourage the onset of pulmonary emphysema in patients with  $\alpha_1$ -at deficiency and, in general, severe grades of emphysema are hardly ever found in non-smokers. The size and number of the lysosomes of the alveolar macrophages is much increased in smokers, and nitrogen dioxide, a constituent of tobacco smoke, is known to promote the release of proteolytic enzymes within the lung. A greater cigarette consumption seems to be required for the production of emphysema in the presence of normal serum  $\alpha_1$ -at than in reduced serum  $\alpha_1$ -at concentration. Many heavy smokers escape emphysema, however, and it seems necessary to postulate that, besides those with  $\alpha_1$ -at deficiency there may be a further group of subjects who are predisposed to develop the disease as a result of cigarette-smoking and of a still undiscovered biochemical abnormality.

Male subjects with  $\alpha_1$ -at deficiency are more likely to develop emphysema than females, and there is a very marked male preponderance among emphysematous patients in general. Research into the pathological causes of primary gout where there is a similar male to female ratio indicates that oestrogenic hormones may stabilize the lysosomal membrane and thus help to prevent release of proteolytic enzymes. Both oestrogens and progesterones have, in fact, been reported to prevent the experimental production of emphysema.

The discovery of the association of  $\alpha_1$ -at deficiency and pulmonary emphysema has opened up many avenues of research, and an understanding of the basic mechanisms responsible for this and other types of emphysema is now possible. The polymorphs and alveolar macrophages, in fulfilling their defensive role may bring about irreversible structural damage to the lung by the release of proteolytic enzymes, and the function of the enzyme inhibitors, of which  $\alpha_1$ -at is one of the most important is evidently to protect the lung from this kind of injury. Disturbance of the balance between the enzymes and their inhibitors by an excess of proteolytic activity may be a crucial factor in the pathogenesis of emphysema, and in  $\alpha_1$ -at deficiency we have a clear example of one way in which this balance may be profoundly altered.

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