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Effects of the protease inhibitor (*Pi*) polymorphism on alpha-1-antitrypsin concentration and elastase inhibitory capacity in human serum

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Summary. The concentration of alpha-1-antitrypsin (AAT) and its elastase inhibitory capacity (EIC) have been investigated *in vitro* in sera from 1688 healthy Canberra blood donors typed for electrophoretic variants of the protease inhibitor (*Pi*) locus. Nine *Pi* alleles were recorded in the sample, of which *M1* was found at a frequency of nearly 70% and the other eight were each at frequencies below 15%. As a class, heterozygotes among the three *Pi M* subtype alleles, *M1*, *M2* and *M3*, have higher means and lower variances for AAT and EIC than do the three *M* subtype homozygotes. Among the three homozygotes *M1M1* has highest AAT and EIC and among the heterozygotes dominance in *M1M2* and *M1M3* is towards or beyond the high *M1M1* values. Of the six other *Pi* alleles recorded, two (*F* and *G*) have similar values to the *M* subtypes but the other four (*I*, *N*, *S* and *Z*) have lower values. The patterns of means and variances in AAT and EIC for the different *M* subtype genotypes do not support the precise threshold function postulated by Martin and Oakeshott (1983) to relate activity to Darwinian fitness. Nevertheless, several aspects of the results are consistent with a general positive relationship between activity and fitness.

1. Introduction

Over the past 25 years, the widespread application of techniques of protein electrophoresis has revealed polymorphic variation at the structural genes of at least one third of human enzymes (Harris 1975). A keen debate has ensued concerning the role of this variation in adaptation and evolution. One way of examining this role is to dissect the physiological and demographic properties of individual polymorphic enzymes and proteins. For haemoglobin and glucose 6-phosphate dehydrogenase, this approach has revealed strong selective relationships between variants deficient in activity and particular disease susceptibilities (see Cavalli-Sforza and Bodmer (1977) for a review).

This approach has recently yielded promising results for a third protein, alpha-1-antitrypsin (AAT), which is the major serum protease inhibitor. Five alleles of the protease inhibitor locus (*Pi*) occur at polymorphic frequencies and these account for much of the variance in serum AAT levels. The alleles are *M1*, *M2*, *M3* (the *M* subtypes), *S*, and *Z*, which have approximate frequencies of 0·75, 0·15, 0·04, 0·04 and 0·01, respectively, in European populations (see Fagerhol and Cox (1981) for a review). The major differences in AAT levels are due to the *S* and *Z* alleles, which produce only about 60% and 10%, respectively, of the 'normal' levels produced by the *M* alleles (Cook 1974). However, much smaller differences also occur among the *M* alleles, with the three *M* subtype heterozygotes (*M1M2*, *M1M3*, *M2M3*) having higher means and lower variances for AAT levels than the three *M* subtype homozygotes (*M1M1*, *M2M2*, *M3M3*) (Beckman and Beckman 1980, Rantala, Finni and Simila 1982).

Several lines of evidence suggest that these differences in AAT levels affect one of its

major functions *in vivo*, namely its elastase inhibitory capacity (EIC). Elastase plays a major role in tissue destruction during respiratory conditions like emphysema and chronic obstructive lung disease (Senior, Tegner, Kuhn, Ohlssen, Starcher and Pierce 1977). EIC activities are highly correlated with AAT levels (Senior, Huebner and Pierce 1971), although specific EIC values for most *Pi* genotypes have yet to be determined (Billingsley and Cox 1980). Many studies have shown greater susceptibility to several respiratory conditions among those genotypes (*MZ, SS, SZ* and *ZZ*) with 60% or less of normal *MM* AAT levels (Fagerhol and Cox 1981, Madison, Mittman, Afifi and Zelman 1981, Horne 1982, Martin, Oakeshott, Clark and Carr 1983).

There is also some evidence for small differences in lung function among *M* subtype genotypes (Gibson, Martin, Oakeshott, Rowell and Clark 1983). Direct evidence that these differences affect susceptibility to respiratory disease has yet to be obtained, but indirect evidence for some effects on Darwinian fitness is provided by the modelling work of Martin and Oakeshott (1983). Using the data of Beckman and Beckman (1980) on means and variances in AAT levels, Martin and Oakeshott (1983) calculated the proportion of each *M* subtype genotype with AAT levels above a threshold of 1·0 mg/ml (about 40% of normal). Then by simply relating fitness to this proportion they were able to solve population genetic equations which predict equilibrium allele frequencies from the relative fitnesses of the different genotypes (Mandel 1959, Crow and Kimura 1970). A stable polymorphism was predicted in which the equilibrium frequencies of the three *M* alleles were all within about 0·02 of the observed values.

Although the general result of this modelling proved robust to variation in the fitness threshold between 0·50 and 1·50 mg/ml, the assumptions in the model obviously oversimplify the biology of the system. Even so, the result is striking in view of the stringency of the mathematical conditions for the stability of a polymorphism, particularly of one adhering so closely to observed frequencies. At the very least, the success of the model justifies careful screening of the *Pi* genotypes, in particular the *M* subtypes, for any direct relationship between EIC, lung function and respiratory disease.

With this aim in mind, however, there are at least three limitations in the activity data presently available for the *Pi* genotypes. First, no reports of EIC activities for *Pi* genotypes have yet been published in which the three *M* subtypes have been distinguished. EIC activity is better than immunological AAT concentration as an indicator of the biological function of alpha-1-antitrypsin; unless the specific activity of AAT molecules is the same for all *Pi* genotypes, then AAT levels will not accurately reflect EIC activities. Second, even for AAT levels, the sample sizes so far measured in the rarer *M* subtype genotypes are too small (e.g. for *M3M3* $N=3$ in Beckman and Beckman (1980) and $N=7$ in Rantala *et al.* (1982)) for reliable estimation of means and variances. Third, no activity data have yet been published for *M* subtype heterozygotes with the low activity alleles *S* and *Z*. Accordingly, we present here the AAT levels and EIC activities of 1688 Canberra blood donors typed for *Pi* and subtyped for *M*.

2. Materials and methods

The subjects were a serial sample of 1688 blood donors (1060 males, 628 females) aged between 18 and 65 years at the Canberra Red Cross Blood Bank. At the time of sampling all subjects satisfied blood donation requirements, so they were free of symptoms of infectious disease and not under medication. Men with haemoglobins below 12·5 g/100 ml and women who were pregnant or had haemoglobins below 11·5 g/100 ml were also excluded.

A blood sample of about 10 ml was taken from each subject and, after an interval of up to six hours at room temperature, was centrifuged at about 2500 g for ten minutes. The supernatant serum was then divided into two equal aliquots which were stored at – 20°C. Between three and six months later, one aliquot was used for determination of *Pi* phenotype and the other for EIC and AAT.

Pi was typed by isoelectric focussing and immunofixation. Isoelectric focussing was carried out on 1 mm thick polyacrylamide gel slabs, containing LKB Ampholine R 3·5–5·0, for two hours at 8°C. The method was that of Arnaud and Chapuis-Cellier (1975) except that maximum voltage was 1800 V and maximum power 35 W. Gels were stained with Coomassie Blue R250 (Sigma). Immunofixation was essentially by the cellulose acetate print method of Arnaud, Wilson, Koistenen and Fudenberg (1977), using DAKO R rabbit antihuman alpha-1-antitrypsin antiserum diluted 1 : 2 with phosphate-buffered saline. Sera were also diluted 1 : 2 with phosphate-buffered saline. Nomenclature of variants was as collated by D. W. Cox from the *Pi* Nomenclature Meeting (Rouen, 1978).

Measurement of AAT and EIC was carried out on a Multistat-3 centrifugal analyser (Instrument Laboratory Inc., Lexington, MA, USA), using serum diluted 1 : 200 in phosphate-buffered saline, pH 7·4. AAT was measured by an immunoturbidometric assay. One hundred microlitres of the prediluted serum was mixed with 200 μl of working antisera (Dako-Immunoglobulins a/s Copenhagen, Denmark), which had been previously diluted, 1 : 20, with 61·8 g/l polyethylene glycol, M.W. 8000, to give a polyethylene glycol concentration in the final reaction mixture of 40 g/l. The reaction was carried out at 37°C, and the turbidity formed after six minutes was measured at 340 nm. Standardization was against 'Protein Standard' and the units were mg/ml (OTFI, Calbiochem-Behring Aust. Pty. Ltd).

EIC was measured using a modification of the method of Klumpp and Bieth (1979). Fifty microlitres of the prediluted serum was incubated for five minutes in the Multistat-3 rotor at 37°C with porcine pancreatic elastase reagent (Calbiochem-Behring Corp., La Jolla, CA, USA). At the completion of the incubation period, the 'free' elastase was measured by a kinetic reaction in which the yellow chromogen *p*-nitroaniline, released from the succinyl-trianiline-*p*-nitroanilide substrate (Calbiochem-Behring), was measured at 410 nm. It was found necessary to increase the concentration of the elastase in the reagent recommended by Klumpp and Bieth by a factor of 1·8. This improved the precision of the method, particularly for low EIC values. EIC was expressed as mg elastase inhibited per ml plasma × units elastase per mg.

3. Results and discussion

Pi *gene and genotype frequencies*

Pi allele frequencies in the Canberra sample are consistent across the two sexes (table 1, $\chi^2_5 = 6{\cdot}97$, $P > 0{\cdot}05$), but differ from those in another large sample of white Australian blood donors from Sydney (Clark 1982). Compared to the Sydney donors, the Canberra sample shows a lower frequency of *M1* (0·69 v. 0·76) and a higher frequency of *M3* (0·08 v. 0·03). The discrepancy may reflect different contributions by various European communities to the two samples.

As was the case in the Sydney sample, genotype frequencies in the Canberra donors are disturbed significantly from Hardy–Weinberg expectations ($\chi^2_{12} = 66$, $P < 0{\cdot}001$). It is formally possible that these discrepancies reflect some mistakes in scoring *Pi* pheno-

types. With our methods it can be difficult to distinguish *M1M3* heterozygotes from blurred *M1M1* or *M3M3* homozygotes. However, we checked all ambiguous samples by repeated testing against known subtypes until consistent typings were obtained in blind runs. We therefore conclude that mistakes in scoring *Pi* type were minimal and that the discrepancies from Hardy–Weinberg expectations were at least in part due to the mixing of different European populations within the sample.

Table 1. *Pi* allele frequencies and observed and Hardy–Weinberg-expected *Pi* genotype numbers in the Canberra sample.

	Allele frequencies								
	M1	*M2*	*M3*	*S*	*Z*	*F*	*I*	*G*	*N*
Males	0·698	0·144	0·083	0·049	0·018	0·002	0·002	0·003	0·001
Females	0·684	0·167	0·085	0·044	0·018	0·000	0·002	0·000	0·000
Total	0·693	0·153	0·084	0·047	0·018	0·001	0·002	0·002	0·000
	Genotype numbers									
	M1M1	*M2M2*	*M3M3*	*M1M2*	*M1M3*	*M2M3*	*M1S*	*M2S*	*M3S*	*SS*
Obs.	853	41	30	328	144	62	113	27	13	2
Exp.	811	40	12	358	197	43	110	24	13	3·7
	M1Z	*M2Z*	*M3Z*	*SZ*	*ZZ*	*M1O**	*M2O*	*M3O*	*(S+Z+O)O*	
Obs.	39	13	4	2	1	8	4	1	3	
Exp.	42	9·3	5·1	2·9	0·5	2·0	0·4	0·2	2·3	

* *O = F + I + G + N*.

Sex differences

Table 2 shows that females have significantly higher means than males for EIC activity, AAT concentration and their ratio (RATIO = EIC/AAT, an indicator of the specific activity of AAT molecules). These sex differences account for only 6%, 5% and 3% of total variation in EIC, AAT and RATIO, respectively (table 3). However, they imply both that females have more AAT molecules and that these molecules have on average a greater biological activity. These differences contrast with the results of Ganrot (1972) and Klumpp and Bieth (1979), who reported similar values in the two sexes, albeit with much smaller sample sizes (< 100). The sex differences we observe are not due to sex differences in allele frequency (table 1) and further analyses not given here show that neither are they due to sex-specific non-linearity in the assays. Nor have co-operative effects between AAT molecules, sex-specific or otherwise, been reported (Fagerhol and Cox 1981).

Table 2. Means, standard deviations and standard errors of EIC, AAT and RATIO (EIC/AAT).†

	EIC			AAT			RATIO		
	$\bar{x}$	SD	SE	$\bar{x}$	SD	SE	$\bar{x}$	SD	SE
Males	14·3	3·5	0·11	2·47	0·35	0·01	5·78	1·00	0·03
	***	***		***	***		***	N.S.	
Females	16·7	4·5	0·18	2·68	0·47	0·02	6·17	1·07	0·04
Total	15·2	4·1	0·10	2·54	0·41	0·01	5·92	1·06	0·03

N.S., Not significant, *** $P < 0·001$.

†The significance of sex differences in means and standard deviations is also shown. EIC and AAT but not RATIO were logarithmically transformed before their significance tests. As in subsequent tables, differences in standard deviations were tested by the method of Bartlett as in Sokal and Rohlf (1969).

The higher mean AAT in females may be associated with elevated levels in women using oral contraceptives (Laurell, Kullander and Thorell 1968). The sex differences in EIC and RATIO might represent true differences *in vivo*, or sex-specific artefacts in the procedures for preparation, storage or assay of the samples. Unlike the immunological AAT protein concentration assay, results from the functional EIC assay depend on the stability and configuration of the alpha-1-antitrypsin molecules. EIC and RATIO could be elevated in females because of other antiprotease molecules which might, like alpha-1-antitrypsin, be present in higher concentrations in that sex. Alternatively, EIC and RATIO could be reduced in males, for example, by higher levels of inactive elastase/alpha-1-antitrypsin complexes (Fagerhol and Cox 1981), the presence of alpha-1-antitrypsin molecules inactivated by cigarette smoke (Dunhill 1979) or differences in stability of EIC activity during storage of the samples.

Table 3. Analysis of variance on logarithmically transformed scores for EIC and AAT and untransformed values of RATIO.†

Source of variation	d.f.	EIC		AAT		RATIO	
		% var.	F	% var.	F	% var.	F
Sex	1	6	132·7***	5	121·2***	3	56·1***
Genotype	23	23	23·6***	26	28·1***	8	6·5***
Sex × genotype	12	1	1·0	1	1·4	1	1·5
Residual	1650						

*** $P<0.001$.

† The percentage of variance explained by, and the F ratio for the effects of sex, *Pi* genotype and their interaction are given.

A regression analysis of EIC against AAT can be informative in this respect (Billingsley and Cox 1980). Additional elastase inhibition due to other protease inhibitors should give a positive EIC intercept at AAT = 0, while reduced inhibition due to instability or inactive complexes should give a negative EIC at AAT = 0. In fact this intercept is negative, more so in males, but not quite significantly so in either sex ($b = -4.67 \pm 2.55$ overall).

In addition to their mean differences, the sexes also differ significantly in variance for AAT and EIC, females having higher variances in both cases (table 2). The variance of RATIO is, however, similar in the two sexes, which indicates that the greater EIC variance of females is due to greater variance in their numbers of AAT molecules, and not in the specific activity of those molecules. The higher variance in numbers of molecules for females, like their higher means, could be related to variation in the use of oral contraceptives.

Before comparing the *Pi* genotypes, it is important to establish that the small sex differences described above are consistent across genotypes. Table 3 shows this to be so. For all three measures the sex × *Pi* gentoype interaction is statistically non-significant and contributes only 1% of total variance. By contrast, main effects of *Pi* genotype are highly significant in all three cases, contributing 23%, 26% and 8% to total variance in EIC, AAT and RATIO, respectively. The negligible interaction terms justify the use of values adjusted for the sex differences in the analysis of the genotypic effects below.

The M *subtypes*

Overall, *M* subtypes in the Canberra sample have comparable mean AAT values to those in previous reports (table 4). However, our values for mean EIC are lower than

Table 4. Means and standard deviations for EIC, AAT and RATIO among *M* subtypes in the Canberra sample and, for AAT, in the Scandinavian samples of Beckman and Beckman (1980) and Rantala *et al.* (1982).†

	Canberra donors				Beckman and Beckman		Rantala *et al.*	
Subtype	*N*	EIC	AAT	RATIO	*N*	AAT	*N*	AAT
M1M1	853	15·8±3·6	2·60±0·36	6·01±0·98	54	2·56±0·49	94	2·83±0·63
M2M2	41	14·2±4·5	2·52±0·51	5·55±1·20	39	2·56±0·56	9	2·61±0·20
M3M3	30	14·9±3·3	2·51±0·28	5·90±1·03	3	2·77±0·81	7	1·79±0·65
Average		15·0±3·8	2·54±0·40	5·82±1·07		2·63±0·64		2·41±0·54
M1M2	328	15·7±3·7	2·60±0·36	5·98±0·99	88	2·86±0·34	45	2·84±0·53
M1M3	144	15·9±3·7	2·62±0·33	6·02±0·97	12	2·81±0·28	26	2·73±0·54
M2M3	62	14·3±3·2	2·48±0·31	5·74±1·11	29	2·66±0·22	9	2·84±0·42
Average		15·3±3·6	2·57±0·33	5·91±1·03		2·78±0·28		2·80±0·50

† Sample sizes (*N*) are also shown. The unweighted averages of the means and standard deviations are given separately for the three homozygous and three heterozygous genotypes. The averages for the standard deviations are actually calculated from the averages of the variances.

those of Klumpp and Bieth (1979) (about 15 v. 25), so our values for mean RATIO are also lower. (Klumpp and Bieth (1979) used about half our elastase concentration and an assay temperature of 30° rather than our 37°C.)

AAT variances in the *M* subtypes are generally lower in the Canberra blood donors than in the samples of Beckman and Beckman (1980; healthy Swedish adults) and Rantala *et al.* (1982; Finnish newborns) (table 4). Given the heterogeneity of age in the Canberra sample, its lower AAT variance may reflect a lower measurement error. Compared to the values of Klumpp and Bieth (1979), the EIC variance in the Canberra sample is lower in absolute terms but comparable in relation to its mean value ($\bar{x}\pm$s.d. = 15±4 in our case and 25±7 in Klumpp and Bieth (1979)).

As a class, *M* subtype homozygotes in the Canberra sample show lower means and higher variances for EIC, AAT and RATIO than the *M* subtype heterozygotes (table 4). To this extent the data agree with the AAT results of Beckman and Beckman (1980) and Rantala *et al.* (1982). However, the differences are much smaller in the present than the earlier surveys. Moreover, the patterns of differences both among the three homozygous genotypes and among the three heterozygotes also differ between the three data sets. The precise activity relationships in the Beckman and Beckman (1980) data which Martin and Oakeshott (1983) found could predict stable *Pi* polymorphism around the observed allele frequencies clearly do not occur in either of the other data sets.

On the basis of its much larger size, the present sample must be considered more reliable. And application of the threshold activity-fitness functions of Martin and Oakeshott (1983) to the Canberra data does not predict stable polymorphism among the *M* alleles. This is true for AAT thresholds throughout a range between 0·50 and 1·50 mg/ml and for EIC and RATIO throughout ranges of equivalent values. It also holds for both untransformed and logarithmically transformed values of all three variables. The *M2* allele (the homozygote for which has the highest variances) is lost in all the solutions and monomorphism is established for either *M1* or *M3*, depending on the variable and threshold considered.

The Canberra sample shows significant differences among the six *M* genotypes in the means and variances of all three measures but much of this variation is due to the *M2M2* homozygote and *M2M3* heterozygote (table 4). Thus the three homozygotes are

homogeneous in mean AAT ($F_{(2,921)} = 3 \cdot 00$, $P > 0 \cdot 05$) but heterogeneous in mean EIC ($F_{(2,921)} = 6 \cdot 69$, $P < 0 \cdot 01$) and mean RATIO ($F_{(2,921)} = 4 \cdot 22$, $P < 0 \cdot 05$), for which *M2M2* has lower values than the other two genotypes, particularly *M1M1*. This suggests that the specific activity of *M2* AAT molecules is lower than those of the other subtype molecules, although the numbers of those molecules are equivalent.

The contributions to measured activity made by the two *Pi* subtypes present in each heterozygote can be influenced by dominance effects. Figure 1 shows this graphically in terms of the means and standard errors of the AAT protein concentration and EIC activity of each *M* subtype homozygote and heterozygote. It appears that for both AAT and EIC two of the heterozygotes, *M1M2* and *M1M3*, are much closer to *M1* than to their other component, *M2* or *M3*. Such dominance suggests some interaction in the control of synthesis or secretion rates, or in the rate of removal of alpha-1-antitrypsin molecules from the circulation. The third subtype heterozygote, *M2M3*, has the lowest activity values of all three heterozygotes and this is consistent with *M3M3* and, particularly, *M2M2* having the lowest values among the three homozygotes. However, there were insufficient differences between the two *M2* and *M3* homozygotes for any meaningful analysis of dominance effects in the *M2M3* heterozygote.

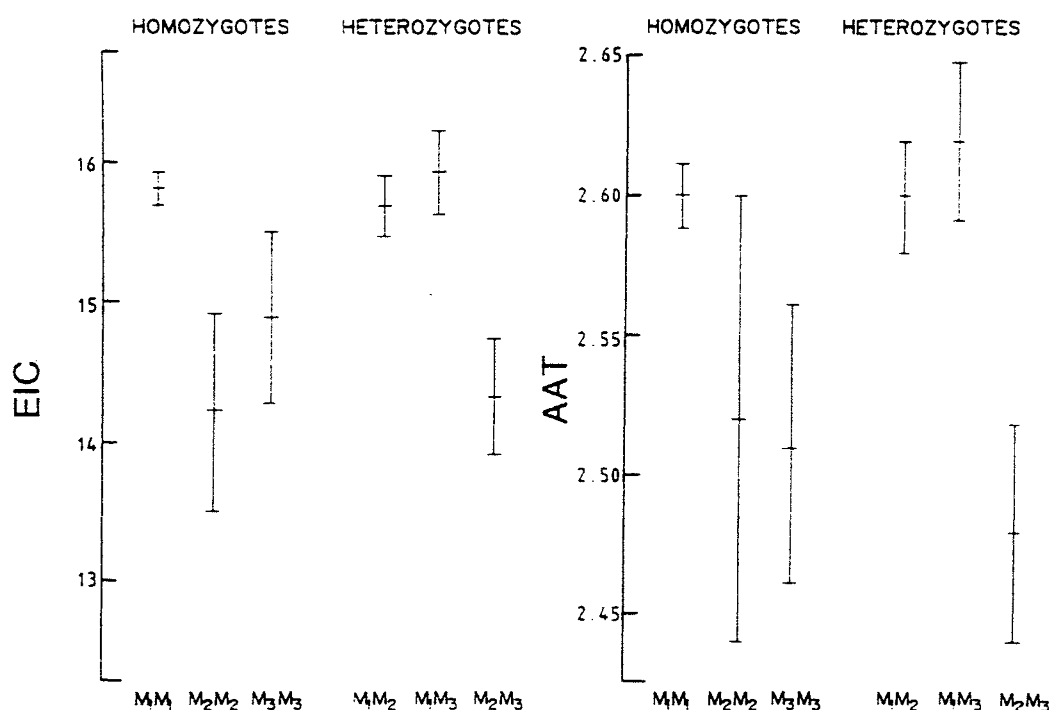

Figure 1. Means and standard errors of EIC and AAT for the *M* subtypes in the Canberra sample.

The S *allele*

The three heterozygous genotypes between the *M* subtypes and *S* are homogeneous in the means and variances of all three measures (except for the variance of RATIO which is significantly lower in *M1S* than *M2S* or *M3S*; table 5). Although the difference is not significant, the fact that *M2S* has the lowest means for EIC and RATIO is again consistent with the differences of the *M2M2* from the *M1M1* and *M3M3* homozygotes (table 4).

Table 5. Means, standard deviations and sample sizes for EIC, AAT and RATIO among *S* genotypes in the Canberra sample. *F* ratios testing the significance of differences in means and standard deviations among various genotypes are also given.

Genotype	*N*	EIC	AAT	RATIO
M1S	113	14·0 ± 3·1	2·32 ± 0·31	5·97 ± 0·94
M2S	27	13·2 ± 4·1	2·21 ± 0·32	5·90 ± 1·45
M3S	13	14·2 ± 3·8	2·21 ± 0·30	6·37 ± 1·48
SS	2	7·4 ± 4·0	1·87 ± 0·07	3·89 ± 1·97
F ratios				
M1S v. *M2S* v. *M3S*		1·1 1·5	2·3 0·7	0·9 6·2**
MS v. *MM*		35·9*** 0·2	115·5*** 0·2	0·0 2·6
SS v. *MS*		14·2*** 2·0	4·4* 1·3	7·1** 0·8

* $P<0·05$, ** $P<0·01$, *** $P<0·001$.

Compared to the six *M* subtype genotypes, the three *MS* heterozygotes have similar variances for all three measures, similar means for RATIO, but lower means (~ 90%) for EIC and AAT (table 5). This suggests that the *S* allele produces fewer AAT molecules than the *M* alleles, but that on average the specific activities of the molecules are comparable. A similar conclusion was reached by Jeppsson, Laurell and Fagerhol (1978).

It therefore seems anomalous in our data that *SS* homozygotes have significantly lower means than *MS* heterozygotes for RATIO as well as EIC and AAT (table 5). (The variances in all three cases are similar.) The discrepancy may be due to our relatively high mean for AAT in *SS* (75% of *M* levels, *c.f.* 60% in Fagerhol (1969)). The reliability of our figure is, however, doubtful since only two *SS* individuals were found in our sample.

Rarer genotypes

The Canberra sample included five alleles at very low frequencies (*Z, G, I, F* and *N*; table 1), usually as heterozygotes with the *M* alleles. No significant activity differences were detected between heterozygotes of each rare allele with the different *M* subtypes (although the analyses had very low power with such small sample sizes). Therefore the data for heterozygotes with the different *M* alleles have been pooled (table 6).

MZ individuals have significantly lower values (60–80%) than the *M* subtypes for EIC, AAT and RATIO. The low EIC is thus explained partly by lower numbers of molecules (AAT) and partly by lower specific activities (RATIO). The lower numbers of molecules are consistent with evidence that the *Z* protein is released more slowly into plasma from the hepatocytes where it is produced, while the lower estimate of specific activity is consistent with evidence of its lower stability *in vivo* (Laurell, Nosslin and Jeppsson 1977). The Canberra sample only included one *ZZ* and two *SZ* individuals; as expected, their values for all three measures were lower even than those for *MZ*.

Both in homozygotes and heterozygotes with *M*, the *G* protein gave values of AAT, EIC and RATIO only trivially different from those of the *M* subtypes. The *I, F* and *N* alleles were only found as heterozygotes with *M*, and *MI* and *MF* individuals generally had slightly but not significantly lower activity values. The lower values for *MI* at least may be meaningful since Arnaud Chapuis-Cellier, Vittoz and Fudenberg (1978) also recorded *I* as having lower AAT levels than *M*. The one *MN* individual in the Canberra sample had significantly lower values for EIC and RATIO but not AAT, which suggests that the *N* protein may have lower specific activity but similar numbers of molecules to *M*.

Table 6. Means, standard deviations and sample sizes for EIC, AAT and RATIO among *Z* and rarer genotypes in the Canberra sample. *F* ratios testing the significance of differences in means among various genotypes are also given.

	N	EIC	AAT	RATIO
MZ	56	8·5±3·4	1·81±0·35	4·59±1·21
SZ	2	4·6±1·5	1·08±0·10	4·33±1·78
ZZ	1	3·4	0·97	3·54
MG	1	14·3	3·03	4·68
GG	3	15·2±2·8	2·46±0·54	6·22±0·92
MI	7	13·9±2·8	2·50±0·28	5·55±1·11
MF	4	13·9±2·4	2·59±0·33	5·31±0·99
MN	1	8·6	2·32	3·71
F ratios				
MZ v. *MM*		374·05***	386·14***	102·49***
MG v. *MM*		0·07	1·46	1·70
MI v. *MM*		1·42	0·44	1·26
MF v. *MM*		0·88	0·00	1·80
MN v. *MM*		5·49*	0·55	5·16*

* $P<0·05$, *** $P<0·001$.

4. Conclusions

While originally supported by the limited data of Beckman and Beckman (1980), the precise threshold activity/fitness model of Martin and Oakeshott (1983) fails comprehensively when applied to EIC and AAT data in the much larger Canberra sample. Under the model the Canberra data do not predict any stable polymorphic equilibria for the three *M* subtypes, let alone one in which equilibrium allele frequencies agree with those observed. Since the pattern of means and variances in the data of Beckman and Beckman (1980) was also not repeated in another small sample of Rantala *et al.* (1982), the Martin and Oakeshott model as strictly proposed must be rejected. Nevertheless, a less specific model of a positive relationship between fitness and activity is still supported by two general aspects of the activity data.

First, in a broad sense, the total data from all three studies (Beckman and Beckman 1980, Rantala *et al.* 1982, and the present survey) confirm that the three *M* subtype heterozygotes as a group do have higher means and lower variances than the group of three *M* subtype homozygotes (table 4). This general result is consistent with better homeostatic buffering among the heterozygotes and implies that smaller proportions of the heterozygotes than homozygotes will be in the low activity range.

Second, it is generally true in our data that the low activity *Pi* genotypes occur at lower frequencies than those with higher activities. Aside from the *M* subtypes, six alleles occurred at low frequencies in the Canberra sample and four of these (*I, N, S* and *Z*) were found to have lower activities than *M*. Even within the *M* subtypes, *M2* and *M3* have lower activities than *M1* and are also much less frequent.

Taken together with the known disease associations of the low activity *S* and *Z* genotypes and the superior lung function of the high activity *M* subtype heterozygotes (see *Introduction*), these two general features of the activity data suggest that a positive relationship between AAT activity and Darwinian fitness may in part determine *Pi* allele frequencies. However, we now also conclude that this relationship is considerably more complex than the simple threshold model proposed by Martin and Oakeshott (1983). We therefore close with a brief consideration of two factors which may introduce such complexity and on which further work is clearly needed if any action of natural selection on the *Pi* polymorphism is to be fully understood.

One potentially complicating factor relates to the fact that the low activity *S* and *Z* alleles, while uncommon (0·05 and 0·02, respectively, in the Canberra sample), are still at frequencies too high to be explained by recurrent mutation alone. For this reason, several authors have suggested that the decreased viability of *S* and *Z* genotypes implied by their disease associations may to some extent be offset by increased fertility. Alpha-1-antitrypsin occurs in cervical mucus (Schumaker 1970), where, it has been suggested, low AAT levels may lead to greater protease activity on the ovum and greater likelihood of penetration by spermatozoa (Fagerhol and Cox 1981, Martin and Oakeshott 1983). Indeed, three studies have reported an increased frequency of the *S* allele in samples of twins and their parents (Cook 1975, Lieberman, Borhani and Feinleib 1978, Clark and Martin 1982). Early results suggested that the *Z* allele may also be preferentially transmitted (at least in males) but more comprehensive analyses of larger samples by Fagerhol and Cox (1981) have not substantiated these findings. Increased fertility among *S* and *Z* genotypes thus remains an intriguing possibility but, for *Z* in particular, has yet to be firmly established.

A second complication in interpreting the AAT activity variation in terms of fitness is that AAT levels are highly inducible in response to inflammation and infection (Fagerhol and Cox 1981). Therefore, the steady-state levels of the different genotypes may be less relevant to their fitness than their inducible levels. The two levels may well be highly correlated across genotypes, but evidence to test this is presently lacking. In this respect we note that the *Pi* locus only explains about a quarter of the variance in AAT levels (table 3); in addition to the 'environmental' effects associated with inflammation and infection, genetic determinants other than *Pi* may contribute to the remaining three quarters.

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Zusammenfassung. Die Konzentration von Alpha-1-Antitrypsin (AAT) und seine Elastase-Inhibitionskapazität (EIC) wurden *in vitro* untersucht bei Seren von 1688 gesunden Blutspendern in Canberra, die auf elektrophorethische Varianten des Locus Protease-Inhibitor (*Pi*) typisiert wurden. Neun *Pi*-Allele wurden in der Stichprobe festgestellt, von denen *M1* in einer Frequenz von fast 70% gefunden wurde und die anderen acht in Frequenzen von weniger als 15%. Als Klasse haben die Heterozygoten der drei *Pi M*-Subtypenallele *M1*, *M2* und *M3* höhere Mittelwerte und niedrigere Varianzen von AAT und EIC als die drei *M*-Subtypen-Homozygoten. Unter den drei Homozygoten hat *M1M1* höchste AAT und EIC und bei den Heterozygoten beläuft sich die Dominanz bei *M1M2* und *M1M3* gegen oder jenseits der hohen Werte von *M1M1*. Von den sechs anderen berichteten *Pi*-Allelen haben zwei (*F* und *G*) ähnliche Werte wie die *M*-Subtypen, wohingegen die anderen vier (*I*, *N*, *S* und *Z*) niedrigere Werte haben. Das Muster der Mittelwerte und Varianzen von AAT und EIC für die verschiedenen Genotypen der *M*-Subtypen unterstützt nicht die genaue Schwellenfunktion, die von Martin und Oakeshott (1983) postuliert wurde, um Aktivität mit Darwinscher Fitneß zu verknüpfen. Dennoch stimmen verschiedene Aspekte der Ergebnisse mit einer allgemeinen positiven Verknüpfung zwischen Aktivität und Fitneß überein.

Résumé. La concentration d'alpha-1-antitrypsine (AAT) et sa capacité d'inhibition de l'élastase (EIC) ont été étudiées *in vitro* dans des sérums de 1688 donneurs de sang bien portants de Canberra typés pour les

variantes électrophorétiques du locus inhibiteur de Protéase (*Pi*). Neuf allèles *Pi* ont été notés dans l'échantillon, parmi lesquels *M1* a été trouvé à une fréquence d'environ 70%; les huit autres avaient chacun une fréquence inférieure à 15%. En tant que classe, les hétérozygotes formés par les allèles *M1*, *M2* et *M3* des sous-types de *Pi M* ont des moyennes plus élevées et des variances plus basses pour AAT et EIC que les trois homozygotes des sous-types de *M*. Parmi les trois homozygotes, *M1M1* a les AAT et EIC les plus élevées, et parmi les hétérozygotes la dominance chez *M1M2* et *M1M3* s'oriente vers les hautes valeurs *M1M1* ou les dépasse. Des six autres allèles *Pi* notés, deux (*F* et *G*) ont des valeurs semblables à celles des sous-types de *M*, mais les quatre autres (*I*, *N*, *S* et *Z*) ont des valeurs plus basses. La configuration des moyennes et variances de AAT et EIC pour les différents génotypes des sous-types de *M* n'étaye pas la fonction à seuil précis postulée par Martin et Oakeshott (1983) comme mettant en relation l'activité à la fitness darwinienne. Quoi qu'il en soit, plusieurs aspects des résultats sont cohérents avec une relation générale positive entre activité et fitness.