homozygotes in each group. Among smokers, genotype would determine whether a subject remained asymptomatic and lost a moderate amount of ventilatory capacity, or developed bronchitic symptoms and lost a good deal more. On such an all-or-none basis for symptoms the amount and duration of smoking would cease to be a prime factor as in the present and in Higgins's data. Among ex-smokers the incidence of cough plus sputum was the same as that among smokers, but the incidence of cough alone was much less; it is suggested on this basis that the persistence or otherwise of symptoms in ex-smokers depends on their relative severity.

At present this hypothesis is offered as a speculative but not unsupported alternative to a direct and quantitative deleterious effect of tobacco smoking on the bronchial mucosa. It appears to merit further investigation.

Summary

Ventilatory capacity has been assessed in 302 subjects in Sydney in relation to smoking habits and respiratory symptoms. Among males, smoking, in the absence of symptoms, leads to some reduction of ventilatory capacity. The presence of cough or cough and sputum is associated with further impairment of ventilatory function. These same symptoms are associated with loss of ventilatory capacity among non-smokers. The ventilatory loss appears to be largely reversible in those who cease smoking. Among females there are similar trends, but the results are not nearly so clear-cut.

The data are not apparently explicable on the basis of a simple quantitative effect of smoking on the bronchial mucosa. As alternative hypothesis based on a combination of genetic and environmental factors is offered, and this alternative hypothesis is supported by the present findings.

We are grateful for helpful statistical comments on this work by Sir Austin Bradford Hill during his visit to Sydney, and for comments about the use of the Wright peak flow meter from Dr. C. M. Fletcher. Dr. Keith Cotton offered valuable criticism of the manuscript. This work was supported by grants from the National Health and Medical Research Council, the Joint Coal Board of N.S.W., the Postgraduate Medical Foundation, and the Consolidated Medical Research Fund of the University of Sydney.

APPENDIX

Among males, assuming random genetic distribution among smokers and non-smokers, and using the groupings in "Discussion":

Incidence of ? VV homozygotes = 0.044 (2/46)
Incidence of ? vv homozygotes = 0.571 (52/91)
Incidence of ? Vv heterozygotes = 0.385 (by subtraction)

If these proportions obtained throughout the whole group of smokers and non-smokers (137) we would have 6.0 ? VV subjects, 52.7 ? Vv subjects, and 78.3 ? vv subjects.

On the basis of the Hardy-Weinberg law (Stern, 1949), we may calculate the expected number in each group if they follow the postulated genetic pattern, using the observed incidence in the ? homozygous groups and the ? heterozygous group to calculate allele frequencies. On this basis, the allele frequency of V (p) is 0.236 ($VV + \frac{1}{2}Vv$) and of v (q) is 0.764 ($vv + \frac{1}{2}Vv$). The expected frequencies of VV (p^2), Vv ($2pq$), and vv (q^2) subjects are thus 0.056, 0.361, and 0.584 respectively, and the expected numbers among 137 subjects become 7.6 VV, 49.4 Vv, and 80 vv. These correspond closely with the observed figures above, and on a statistical basis do not differ significantly from them ($\chi^2 = 0.5933$; $n = 2$; $P = 0.7-0.8$). As a further indica-

tion that the observed incidences would closely fit such a genetic hypothesis, we may apply the criterion that $\sqrt{VV} + \sqrt{vv}$ should equal 1. In this case they equal 0.964, a disagreement of less than 4% *pro rata*.

A similar measure of agreement may be calculated for the female data. The allele frequencies for females become 0.275 for V and 0.725 for v, not very different from the calculated male frequencies.

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TOBACCO SMOKING AND SERUM LIPIDS IN OLD MEN

BY

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The interrelationships between coronary heart disease, serum lipids, and cigarette-smoking have been demonstrated by authors from several countries in recent years (Hammond and Horn, 1954; Gertler and White, 1954; Gofman *et al.*, 1955; Doll and Hill, 1956; Thomas, 1958; Karvonen *et al.*, 1959; Dawber *et al.*, 1959; Bronte-Stewart, 1961). Their work has, however, been chiefly concerned with men below or during middle age, whereas it seems that over the age of 65 years there is little relationship between death from coronary heart disease and a previous history of smoking (Doll and Hill, 1956) or between clinical evidence of myocardial ischaemia and raised serum cholesterol (Oliver, 1958; Acheson, 1961). It is therefore a matter of some interest whether serum lipids are related to smoking habits in men aged 65 and over.

Material and Method

The Sample.—The men were all pensioners on the list of Messrs. Arthur Guinness Son and Company Ltd. (Dublin), and their names were selected in a random manner as follows: every man aged between 75 and 85 was chosen, between the ages of 70 and 74 every third name was omitted from the list, and between the ages of 65 and 69 alternate names were omitted. This method of selection produced 267 names in all, and each man was sent a postcard inviting him to attend in the company's medical department for clinical examination; the actual attendances (221) are shown

by age in Table I, together with the numbers for whom estimations of serum cholesterol and an analysis of the serum proteins by paper electrophoresis were satisfactory.

TABLE I.—*The Sample*

Age in Years	Number of Men						
	Invited to Attend	Attended		With Complete Cholesterol Assessments		With Complete Serum Electrophoresis	
		No.	%	No.	%	No.	%
65-69	92	82	89	74	80	72	78
70-74	100	86	86	83	83	77	77
75-79	40	29	72	29	72	29	72
80-85	35	24	69	23	66	23	66
Total	267	221	82	209	78	201	75

Clinical Examination and Lipid Estimations.—Full details have already been published of clinical procedures (Acheson and Acheson, 1958; Acheson, 1961) and of biochemical and electrophoretic techniques (Acheson *et al.*, 1958; Acheson and Jessop, 1961); accordingly only an outline will be given here. The clinical examination, which was carried out by one of us (R. M. A.), included a history of smoking habits and a note about diet that permitted a rough appraisal of whether or not these old men fed themselves adequately. Blood was taken in a dry syringe between 9 a.m. and 11 a.m., after breakfast, and within 90 minutes the serum was separated by centrifuging. The serum cholesterol was estimated by three independent observers, one of whom used the method of Sackett (1925) and two that of Anderson and Keys (1956); and since the findings by the two methods did not differ systematically the results were averaged, when the three estimations were within 10% of the highest of the three. In the relatively few cases where they were not, assessments were repeated until the required agreement level was reached. Paper electrophoresis, with certain minor modifications, was carried out by the technique of Dangerfield and Smith (1955), using a horizontal EEL apparatus and a barbitone buffer (pH 8.6). Each strip was divided in two, light green S.F. ("lissamine" green) being used to stain the serum proteins and sudan black for the lipoproteins. The intensity of staining in the various bands was measured with an automatic reflectance scanner which incorporated its own integrator.

Tobacco and Dietary Indices.—For certain of the calculations tobacco and dietary indices have been used. For the tobacco index 1 oz. (28 g.) of tobacco a week and 5 cigarettes a day were taken as unity. Thus a man who smoked 20 cigarettes a day had an index value of 4, and another who smoked 10 cigarettes a day and 3 oz. (85 g.) of pipe tobacco a week had a value of $2+3=5$. The dietary index simply enumerated the number of times a man ate meat, fish, or egg in a week; it ranged from 0 to 19, and, although it is obviously a very rough measure, the serum albumin was significantly higher in those with an index of 14 than in those with an index of less than 2; and men with an index of 2 or under had significantly less subcutaneous adipose tissue than the remainder of the sample (Acheson and Jessop, 1961).

Results

Relationship of Serum Cholesterol and Smoking With Age

The distribution of serum cholesterol in the sample is shown by five-year age-groups in Fig. 1 and that of β/α lipoprotein ratio in Fig. 2. There tended

to be absolutely and relatively fewer men with a serum cholesterol in excess of 220 mg. among the older age-groups, with the result that the mean value fell slightly

FIG. 1.—Histograms showing distribution of serum cholesterol in sample by five-year age-groups.

FIG. 2.—Distribution of β/α lipoprotein ratio by five-year age-groups.

but significantly with age ($r = -0.19$; $P < 0.01$). The pattern of smoking varied in a far more striking manner with age (see Table II); 66% of the men aged 75 years and over were pipe smokers and 9% cigarette smokers, as compared with 27% pipe smokers and 53% cigarette smokers in those who were less than 75.

TABLE II.—*Smoking Habits by Age Among Men with Cholesterol Assessments*

Type of Tobacco Used	Age in Years			
	Under 75		75 and Over	
	No.	%	No.	%
None ..	26	17	12	23
Cigarette only ..	83	53	5	9
<20 day ..	45	29	2	4
>20 day ..	38	24	3	5
Pipe only ..	42	27	35	66
Cigarette and pipe ..	5	3	1	2
Total ..	156	100	53	100

Relationship Between Serum Lipids and Smoking

The nature of the relationship between smoking and both serum cholesterol and β/α lipoprotein ratio is indicated in Table III. This shows that both measures of serum lipid are just a little higher in non-smokers or very light smokers than in the heavier smokers of cigarette or pipe tobacco. This trend is confirmed by the correlation analysis shown in Table IV, where it

TABLE III.—*Relationship of Cigarette and Pipe Smoking with Serum Cholesterol and β/α Lipoprotein Ratio*

Type of Tobacco Used	Mean Serum Cholesterol (mg.)		Mean β/α Lipoprotein Ratio	
	No.	Mean	No.	Mean
None	38	214	36	2.0
Cigarette only; No./day:				
5 ..	12	201	11	2.1
10 ..	34	213	33	1.9
20 ..	33	201	33	1.9
30 and over ..	8	206	8	1.8
Pipe only; oz./week:				
1 ..	3	203	3	2.2
2 ..	25	197	23	1.7
3 ..	21	213	19	1.9
4 ..	17	211	17	1.8
5 and over ..	11	217	11	1.7

can be seen that all the coefficients of correlation are negative, but with the exception of β/α lipoprotein ratio in pipe smokers and non-smokers the trends are not statistically significant.

TABLE IV.—*Correlation Between Serum Lipids and Smoking in the Entire Sample*

	Serum Cholesterol (mg./100 ml.)	Serum Lipoproteins β/α Ratio
Cigarette smokers and non-smokers ..	-0.07	-0.10
Pipe smokers and non-smokers ..	-0.14	-0.33*
Tobacco index ..	-0.01	-0.11

* $P < 0.001$; none of the other statistics are significant at the 5% level.

Bronte-Stewart (1961) believes that the proved relationship between serum cholesterol and cigarette-smoking in younger men may be due to dietary preferences. It is possible that these old people living on pensions cannot afford to smoke heavily and to eat well, and therefore a comparison was made between the dietary indices and some other characteristics of, on the one hand, the heavy smokers of both cigarettes and pipe tobacco and, on the other, the non-smokers (Table V). It can be seen that the heavy cigarette-smokers are significantly younger than the other two groups—a finding which reflects the difference between the smoking habits of the generation which reached maturity before and after 1914. In the sample as a

TABLE V.—*Serum Lipids, Age, and Dietary Index of Non-smokers and Heavy Smokers of Cigarette and Pipe Tobacco*

	No.	Age in Years		Cholesterol (mg.)		β/α Lipoprotein Ratio		Dietary Index	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Non-smokers	38	72.4	5.1	214.5	42.9	2.0	0.4	7.1	3.6
30 cigs./day or more	8	67.7*	1.9	205.7	37.3	1.8	0.3	7.0	2.3
5 oz. pipe tobacco/week or more ..	11	72.3	4.1	216.8	65.0	1.7	0.3	9.4	4.9

* Significantly younger than the other two groups ($P < 0.02$); no other group differences are significant at the 5% level.

whole there was a significant negative regression of serum cholesterol with age of 1.7 mg./year (Acheson *et al.*, 1958), but, even allowing for this, the differences between the mean serum cholesterol values are not significant at the 5% level. The dietary index used in this study does not measure preferences, but it has already been shown above that there is reason to believe that it measures the general level of nutrition and therefore would show whether the heavy smokers go without food. The analysis in Table V indicates that this is not the case; indeed, the heavy pipe-smokers eat rather more than the non-smokers, although the difference is not quite significant ($t = 1.69$; $0.1 > P > 0.05$).

Discussion

There can be little question of the relationship between serum lipids and cigarette-smoking in middle-aged men. It has been demonstrated in the U.S.A. (Gofman *et al.*, 1955; Thomas, 1958; Dawber *et al.*, 1959), Finland (Karvonen *et al.*, 1959), and South Africa (Bronte-Stewart, 1961), yet in the present data based on old men there is no evidence whatsoever of such a relationship—in fact, there is a suggestion that non-smokers may have a mean serum cholesterol and β/α lipoprotein ratio which are higher than in smokers.

Bronte-Stewart (1961) believes that the reason serum lipids are raised in younger smokers is because taste preferences induce them to eat more cholesterologenic

substances, such as saturated fats, than non-smokers: he has published some evidence, which he does not claim to be conclusive, to support his view (Bronte-Stewart, 1961; Perrin *et al.*, 1961).

The lack of correlation between serum lipids and smoking habits in the present sample receives indirect support from the fact that smoking does not seem to be associated with coronary heart disease in them (Acheson, 1961) or in two other samples of men over the age of 65 years (Hammond and Horn, 1954; Doll and Hill, 1956). On the present evidence it is not possible to give a reason for this, even less to explain the possibility of an inverse relationship, but several theories suggest themselves. First, as has been stated, limited financial means might have caused those who smoke more to eat less, but in the present sample there was nothing to suggest that means were so limited nor that heavy smokers ate less. Secondly, if we accept Bronte-Stewart's basic hypothesis, it could reasonably be argued that the effect of ageing on the sense of taste may be more powerful than any effect of tobacco-smoking, so that the latter is obscured among old people. Thirdly, it could be that with increasing age the pattern of metabolism of fats and lipids is changed.

Our finding, although difficult to explain, is compatible with our knowledge of the aetiology of coronary heart disease in old men. In younger age-groups the greater part of coronary disease takes the form of infarction caused by thrombotic occlusion of a major vessel, a lesion which has repeatedly been shown to be associated with high serum cholesterol and a raised β/α lipoprotein ratio. Bronte-Stewart (1961) believes that the relationship between cigarette-smoking and such disease is due to the raised serum cholesterol which smokers have, rather than to any pharmacologically induced response smoking may elicit from the cardiovascular system. Only 9% of the present series had myocardial infarction, and in them the serum cholesterol and β/α lipoprotein ratios were raised; the remaining 81%, the majority of whom had angina pectoris and/or myocardial ischaemia, did not differ from the controls in their smoking habits or their serum lipids or serum cholesterol. Dawber *et al.* (1958), in a longitudinal study of younger men, have shown that, although cigarette-smoking and high serum cholesterol predispose to coronary thrombosis, neither predisposes to new cases of coronary heart disease presenting as angina pectoris. In line with this is the failure of Paterson *et al.* (1956) to demonstrate a correlation between cholesterol levels during life and the extent of atherosclerosis at necropsy. Our present finding, therefore, is consistent with the view already expressed (Acheson, 1961) that coronary heart disease which presents as angina pectoris and electrocardiographic evidence of myocardial ischaemia without infarction in old men is of different aetiology from acute coronary thrombosis, which is the more usual form the disease takes in younger men.

Summary

It is shown in a random sample (221) of a defined population of men aged 65–85 years that tobacco-smoking, whether in pipes or cigarettes, is not associated with elevation of the serum cholesterol or the β/α lipoprotein ratio. The significance of this finding in the aetiology of coronary heart disease is discussed.

We are again pleased to acknowledge our gratitude to the directors of Messrs. Arthur Guinness Son and Co. Ltd.

(Dublin), to Dr. Brian Pringle and Dr. J. F. Eustace, and to our colleagues in the Moyne Institute, Trinity College, Dublin, for making this study possible. Miss Jane Cooper helped with the computations and Dr. Richard Doll kindly read the text.

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METHODS OF DIAGNOSING CERVICAL CARCINOMA-IN-SITU

BY

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All gynaecological patients in our unit since 1951 have been screened by the method of exfoliative cytology. Since 1958, with an increase in the number of technicians able to share the microscopical work, we began to screen all antenatal patients, so that we have now some idea of the place of cytology in clinical practice. The basic use of cytological screening is that it leads to a diagnosis of carcinoma-in-situ which is beyond our usual clinical methods; that is to say, speculum examination shows no sign of the disease at routine gynaecological examination (Papanicolaou and Traut, 1943).

Many clinicians would like to search for carcinoma-in-situ, accepting as we do that it is a curable precursor of invasive carcinoma, but they lack funds to pay for the technical help or the time to carry out the painstaking microscopy on which the success of exfoliative cytology rests. On the other hand, there are clinicians who do not yet screen their patients, since they believe that the case for the removal of carcinoma-in-situ and the subsequent reduction in the incidence of invasive carcinoma in the community has yet to be made out. They concede, however, that for the individual woman with carcinoma-in-situ it is best for her to have it cured at once. There is no clinician in this country or elsewhere who has the temerity to watch known carcinoma-in-situ of the cervix for an indefinite period.

We would refer the clinician who is considering the use of cytology to the excellent studies of Ericson *et al.* (1956) in the U.S.A., and to the even more impressive long-term studies in British Columbia by Fidler, Boyes, and Lock (1957). From the Canadian studies it would seem to us that the case for cytology as an obligatory examination is clearly made out, and Boyes and his colleagues in British Columbia can now offer (via 1,100 family doctors) a cytological test at regular intervals to patients without gynaecological symptoms, and at present 22% of sexually mature women have the test regularly (Boyes, 1961, personal communication). A recent survey of the incidence of invasive carcinoma of the cervix in British Columbia shows a fall of 21%, and this reduction is thought to result from the surgical cure of carcinoma-in-situ. Naturally this fall is unlikely to continue to the complete elimination of cervical carcinoma, but, with cytological tests accepted by more and more women, many will be protected from carcinoma by its removal in its "pre"-stage. Moreover, apart from explosive rapidly growing carcinoma, cytology tests should tend to bring the patient with carcinoma for treatment at an earlier and more curable stage; but this is a less important function of cytology.

From our own experience and with the therapeutic experience of Boyes and his colleagues now available, we feel that it must be accepted that nowadays no gynaecological or obstetrical examination is complete without cytology. The financing of a cytology service for women without symptoms must be discussed in each community and by the National Health Service, for cytology is expensive. It is, however, very cheap when compared with the cost of treating carcinoma with its dreadful mortality and morbidity. Apart from hospital patients, intelligent women in possession of the facts will wish to have the protection provided by cytology. Given time and careful propaganda many women who have come to regard the prophylaxis of antenatal care or even poliomyelitis as commonplace will come to accept cytology "as a routine health examination not really much different from an annual visit to the dentist" (McLennan, 1958).

Our Experience with Cytology

We have already reported our experience of the technique in gynaecological practice (McLaren, Taylor, and Attwood, 1956, 1958) and also when applied to special groups such as diabetics (Noble and Attwood, 1958). We can summarize as follows: from routine cytological screening of gynaecological out-patients we expect (a) cervical carcinoma-in-situ in 3 to 4 per 1,000, (b) carcinoma of cervix missed at routine clinical examination in 1 per 1,000, and (c) carcinoma of the corpus not obvious in out-patients—for example, in menorrhagia in the fourth decade—in about 1 per 1,000. In round figures, 1 in every 200 is a "cytological pick-up"—that is, a diagnosis resulting from reassessment of a patient's condition prompted by the finding of cells regarded by a trained technician as sufficiently like cells from carcinoma or carcinoma-in-situ to justify biopsy studies.

Finally, there are a group of cytology findings which are not yet fully understood but which are at present being studied; the label given to these cell smears is "dyskaryosis," where certain nuclear appearances resemble those found in malignancy. We have evidence that patients who produce this type of cell must be