

TOBACCO SMOKING AND BLOOD GROUP

BY

I. T. T. HIGGINS,* M.D., M.R.C.P.
Medical Research Council's Epidemiological Research
Unit, Cardiff

R. J. DRUMMOND, M.R.C.S., L.R.C.P.

P. D. OLDHAM, M.A., F.S.S.
Medical Research Council's Pneumoconiosis Research
Unit, Cardiff

B. BEVAN, M.B., B.Ch., B.Sc.
Welsh Regional Transfusion Laboratory, Cardiff

It is surprising that practically no work has been done on smoking and the blood groups. One might have expected that the demonstration of similarities between the smoking habits of identical twins (Fisher, 1957; Friberg, Kaij, Dencker, and Jonsson, 1959) and of differences in physique (Diehl, 1929; Parnell, 1951), temperament (Heath, 1958; Lilienfeld, 1959; Eysenck, Tarrant, Woolf, and England, 1960), and liability to accidents (Lowe, 1960) between smokers and non-smokers would release a spate of papers on this topic; but this has not been so.

Cohen and Thomas (1962), however, have shown differences in blood-group frequency associated with smoking. In a study of 1,005 white male blood-donors these workers found a significant excess of blood group B in non-smokers and occasional smokers when pooled and a corresponding deficiency of group B among heavy smokers of cigarettes. They also found a higher proportion of rhesus-negative persons (29.4%) among the small number of occasional smokers. In view of the low level of significance in this study confirmation of the association is clearly desirable. The purpose of this paper is to record our findings in a random sample of miners and ex-miners studied in 1961.

The sample consisted of 1,250 miners and ex-miners aged 35-64 living in the Rhondda Fach, a mining valley in South Wales. The procedure adopted for sampling from the community and the methods used have been described elsewhere (Higgins, Oldham, Kilpatrick, Drummond, and Bevan, 1963). Briefly there were 500 men in each of the two decennial groups 35-44 and 55-64 and 250 men in the 45-54 group. The response rate is shown in Table I.

TABLE I.—Random Sample of Miners and Ex-miners Stratified by Age

Age-group	No. in Population at Census*	No. Sampled	Excluded		Left Area		Dead		Refused		Examined	
			No.	%	No.	%	No.	%	No.	%	No.	%
35-44	1,137	500	5	1.0	27	5.4	1	0.2	27	5.4	440	88.0
45-54	1,263	250	1	0.4	9	3.6	10	4.0	23	9.2	207	82.8
55-64	1,168	500	2	0.4	8	1.6	39	7.8	37	7.4	414	82.8
Total	3,568	1,250	8	0.6	44	3.5	50	4.0	87	7.0	1,061	84.9

* Date of Census June 1, 1958.

Methods

Blood grouping was performed using clotted blood samples, these being tested the day after collection. The methods used were those described by the Medical Research Council (1958) and by Drummond (1961).

1. *ABO Grouping*.—Cells were tested with A, B, and O grouping sera. Sera were tested against A and B cells, and they were also tested for the presence of irregular antibodies—for example, anti-Rh agglutinins. Tests were done to ensure that weakly reacting A agglutinin (A_3 , etc.) in group A or AB bloods did not escape detection. In the case of bloods initially typed as group B, the cells were further tested with group O serum having strong anti-A

*Present address: Graduate School of Public Health, University of Pittsburgh, Pittsburgh 13, Pennsylvania.

and which had been absorbed of its anti-B agglutinin. The sera of bloods typed initially as B were also tested with A_1 and A_2 cells.

2. *Rh Typing*.—Cells were tested with two anti-D sera and those giving negative reactions were further tested with anti-C, anti-D, and anti-E sera. The D^u antigen was tested for by the indirect antiglobulin test after treatment of the cells with potent anti-D serum known to react with the D^u antigen. Bloods classified as D-negative were those giving negative reactions with anti-D and anti- D^u sera. D-negative bloods comprise those which lack the C and E antigens, or which contain one or other, or both, of these antigens.

3. *Saliva Tests*.—Salivas were heated immediately after collection in boiling water for 15 minutes. On receipt at the transfusion centre the cardboard cartons and their contents of bottles of saliva were autoclaved entire at 10 lb./sq. in. (0.7 kg./sq. cm.) pressure for 20 minutes, this being done to eliminate risk of infection of laboratory workers. All salivas were tested independently by two observers for A, B, and H substances. Anti-H extracted from seeds of *Ulex europaeus* was used. One observer used a single-tube technique, with suitably diluted antisera. The other observer titrated the salivas. Any discrepancies between the results obtained were checked by repeating tests and, as need be, fresh saliva samples were obtained.

Smoking Habits.—These were recorded by a single interviewer using a standardized questionnaire similar to that approved in 1960 by the Medical Research Council's Bronchitis Committee. The sample was classified into non-smokers, smokers, current smokers, and ex-smokers. A non-smoker is defined as one who has never, at any period of his life, smoked as much as 1 g. of tobacco a day for one year.* In accord with this definition non-smokers have been subdivided into those who have never smoked at all and those who have had an occasional smoke. Current smokers of cigarettes have been differentiated from smokers of pipes and cigars and classified, according to the amount smoked, into smokers of 1-14, 15-24, and 25 g./day and over.

Results

The frequency of the blood group B among the non-smokers was slightly above the average for the whole group of smokers (Table II), but the difference is quite insignificant ($P \triangleq 0.3$). The significance is similar when ex-smokers are counted with smokers and when the comparison with cigarette smokers only. The significance is enhanced slightly if the occasional smokers (those who smoke less than 1 g./day) are removed from the non-smokers and counted with the smokers ($P \triangleq 0.16$).

Table II also shows that variation of smoking habits with the rhesus blood group and with secretor status is negligibly small. If the non-smokers are divided into those who never smoked and those who smoke occasionally, the excess of Rh-negative persons found in the latter group by

*One cigarette is equivalent to 1 g. of tobacco.

TABLE II.—*ABO and Rhesus Blood-group Frequency and Secretor Status According to Smoking Habits*

Blood Group	Total		Smoking Habits																				Total	Not Recorded		
			Non-smokers						Current Smokers																Ex-smokers	
			Never Smoked		Occasion-ally Smoked*		Total		Cigarette g. Tobacco/Day								Other (Pipe and Cigar)		Total		No.	%				
			No.	%	No.	%	No.	%	1-14		15-24		25+		Total		No.	%	No.	%						
O ..	460	43.4	38	42.2	14	42.4	52	42.3	133	40.7	117	46.8	39	39.0	289	42.7	54	45.0	343	43.0	64	46.7	459	43.4	1	
A ..	444	41.8	35	38.9	13	39.4	48	39.0	151	46.2	94	37.6	44	44.0	289	42.7	48	40.0	337	42.3	57	41.6	442	41.8	2	
B ..	113	10.7	14	15.6	3	9.1	17	13.8	27	8.3	33	13.2	10	10.0	70	10.3	12	10.0	82	10.3	13	9.5	112	10.6	1	
AB ..	44	4.1	3	3.3	3	9.1	6	4.9	16	4.9	6	2.4	7	7.0	29	4.3	6	5.0	35	4.4	3	2.2	44	4.2	0	
Rh pos. ..	880	82.9	71	78.9	29	87.9	100	81.3	273	83.5	218	87.2	80	80.0	571	84.3	100	83.3	671	84.2	106	77.4	877	83.0	3	
„ neg. ..	181	17.1	19	21.1	4	12.1	23	18.7	54	16.5	32	12.8	20	20.0	106	15.7	20	16.7	126	15.8	31	22.6	180	17.0	1	
Secretor pos. ..	777	73.2	66	73.3	23	69.7	89	72.4	236	72.2	183	73.2	78	78.0	497	73.4	85	70.8	582	73.0	104	75.9	775	73.3	2	
„ neg. ..	282	26.6	24	26.7	10	30.3	34	27.6	91	27.8	67	26.8	22	22.0	180	26.6	34	28.3	214	26.9	33	24.1	281	26.6	1	
No saliva ..	2	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	0.8	1	0.1	—	—	1	0.1	1	
Total	1,061		90		33		123		327		250		100		677		120		797		137		1,057		4	

* Less than 1 g./day for one year.

Cohen and Thomas (1962) is not present. Indeed, the occasional smokers have a higher proportion Rh-positive than any other group (29 out of 33; 88%), though this is far from significant.

The ABO results, though showing no strong evidence for an association with smoking habits, are nevertheless in the same direction as those reported by Cohen and Thomas (1962) in white male donors, save that in their series the occasional smokers shared the excess of group B shown by the non-smokers, while in our series they share the small deficiency of group B shown by the smokers. Table III brings the two sets of results together, in the form of Cohen and Thomas's Table III. If we compare group B with the other groups and use Woolf's method of analysis (Roberts, 1957) we find:

	χ^2	P
Cohen and Thomas	7.808	0.005
Present series	1.294	0.255
Mean	8.399	0.004
Heterogeneity (1 d.f.) ..	0.702	0.402

TABLE III.—*Comparison of Present Series with Cohen and Thomas's (1962) Series; Occasional Cigarette Smokers Classed as Non-smokers*

		Non-smokers and Occasional	Cigarette Smokers
Cohen and Thomas: White males	O	99 (42.1)	284 (47.2)
	A	86 (36.6)	242 (40.2)
	B	37 (15.7)	54 (9.0)
	AB	13 (5.5)	22 (3.7)
	Total	235 (99.9)	602 (100.1)
Present series	O	52 (42.3)	289 (42.7)
	A	48 (39.0)	289 (42.7)
	B	17 (13.8)	70 (10.3)
	AB	6 (4.9)	29 (4.3)
	Total	123 (100.0)	677 (100.0)

Thus the unimpressive association observed in the present series is not in conflict with Cohen and Thomas's data, and when the two sets are combined the significance is quite high.

When, as seems more logical, the "occasional smokers" are classed with smokers rather than with non-smokers, our data and Cohen and Thomas's show very close agreement (Table IV). The analysis is as follows:

	χ^2	P
Cohen and Thomas	3.471	0.063
Present series	2.256	0.135
Mean	5.726	0.017
Heterogeneity (1 d.f.) ..	0.001	0.975

This comparison is still somewhat artificial, in that it is restricted to men who have never smoked and to cigarette smokers. In fact, the "relative incidence" and significance are little changed if the comparison is made between men

TABLE IV.—*Comparison of Present Series with Cohen and Thomas's (1962) Series; Occasional Cigarette Smokers Classed as Cigarette Smokers*

		Non-smokers	Cigarette Smokers (inc. Occasional Smokers)
Cohen and Thomas: White males	O	75 (40.8)	308 (47.2)
	A	73 (39.7)	255 (39.1)
	B	27 (14.7)	64 (9.8)
	AB	9 (4.9)	26 (4.0)
	Total	184 (100.1)	653 (100.1)
Present series	O	38 (42.2)	303 (42.7)
	A	35 (38.9)	302 (42.5)
	B	14 (15.6)	73 (10.3)
	AB	3 (3.3)	32 (4.5)
	Total	90 (100.0)	710 (100.0)

who have smoked in any way and men who have not. Table V shows the figures, including the American negroes in Cohen and Thomas's series, and the analysis is:

	χ^2	P
Cohen and Thomas: White males	3.371	0.066
Negro "	0.412	0.521
Present series	2.510	0.113
Mean	5.992	0.014
Heterogeneity (2 d.f.) ..	0.301	> 0.80

TABLE V.—*Comparison of Present Series with Cohen and Thomas's (1962) Series, Including Smokers of Pipes and Cigars*

		Non-smokers	All Others
Cohen and Thomas: White males	O	75 (40.8)	385 (46.9)
	A	73 (39.7)	322 (39.2)
	B	27 (14.7)	82 (10.0)
	AB	9 (4.9)	32 (3.9)
	Total	184 (100.1)	821 (100.0)
Negroes	O	32 (65.3)	182 (52.9)
	A	4 (8.2)	84 (24.4)
	B	11 (22.4)	64 (18.6)
	AB	2 (4.1)	14 (4.1)
	Total	49 (100.0)	344 (100.0)
Present series	O	38 (42.2)	421 (43.5)
	A	35 (38.9)	407 (42.1)
	B	14 (15.6)	98 (10.1)
	AB	3 (3.3)	41 (4.2)
	Total	90 (100.0)	967 (99.9)

One must conclude, therefore, that while the present series provides no convincing evidence of an association between smoking and the ABO blood groups, it is not in serious conflict with Cohen and Thomas's data, and, combined with it, suggests that an association may exist.

Discussion

Our findings in this sample are essentially negative, and, in the absence of any previous work on smoking and the

human blood groups, we would certainly have suggested that the small differences of blood-group frequency among different sorts of smoker and between smokers and non-smokers were most probably the effect of sampling variation. As it is, our results are not in conflict with those of Cohen and Thomas (1962), and it is still possible, therefore, that an association between blood group B and smoking may exist generally. Most encouraging is the fact that this association may be of the relatively simple form of a contrast between those who have never smoked and those who have, the former showing an excess of group B. Such an excess is present in both negro and white males in Cohen and Thomas's series and in the present series. It is to be hoped that further data will be accumulated by other workers; the fact that the apparent association is with the relatively rare blood group B means that large numbers will be required before chance can be ruled out as its source.

The importance of showing the existence of any differences in blood-group frequency in relation to smoking, as was pointed out in the *British Medical Journal* (1962), is clear. Any such differences could reflect a genetic element in the adoption of different smoking habits or heterogeneity of the population in this respect for other reasons. In either case light might be thrown on the relation of smoking both to lung cancer and to chronic bronchitis by a search for corresponding variations in the apparent risks of these conditions. To date no particular blood group has been found to be associated with the development of lung cancer, and our findings (Higgins *et al.*, 1963) suggest that in miners, at least, neither blood group nor secretor status is related to the respiratory

symptoms on which a diagnosis of chronic bronchitis is usually based, but further observations are desirable.

Summary

ABO and rhesus blood groups, secretor status, and smoking habits have been determined in 85% of a random sample of miners and ex-miners aged 35-64 from the Rhondda Fach. Neither blood group nor secretor status was significantly related to smoking habits. Our findings and those of Cohen and Thomas in respect of the ABO system and smoking do not, however, differ significantly, and further studies are needed to increase the accuracy of the pooled estimate, which at present suggests that the small number of men who have never smoked is half as large again in group B as in other groups.

REFERENCES

- Brit. med. J.*, 1962, **1**, 932.
Cohen, B. H., and Thomas, C. B. (1962). *Bull. Johns Hopk. Hosp.*, **110**, 1.
Diehl, H. S. (1929). *Minn. Med.*, **12**, 424.
Drummond, R. J. (1961). *Extra Pharmacopoeia (Martindale) Supplement*, p. 93. Pharmaceutical Press, London.
Eysenck, H. J., Tarrant, M., Woolf, M., and England, L. (1960). *Brit. med. J.*, **1**, 1456.
Fisher, R. A. (1957). *Ibid.*, **2**, 297.
Friberg, L., Kaij, L., Dencker, S. J., and Jonsson, E. (1959). *Ibid.*, **1**, 1090.
Heath, C. W. (1958). *Arch. intern. Med.*, **101**, 377.
Higgins, I. T. T., Oldham, P. D., Kilpatrick, G. S., Drummond, R. J., and Bevan, B. (1963). To be published.
Lilienfeld, A. M. (1959). *J. nat. cancer Inst.*, **22**, 259.
Lowe, C. R. (1960). *Brit. J. prev. soc. Med.*, **14**, 57.
Medical Research Council (1958). Memorandum No. 36. H.M.S.O., London.
Parnell, R. W. (1951). *Lancet*, **1**, 963.
Roberts, J. A. F. (1957). *Brit. J. prev. soc. Med.*, **11**, 107.

DIURETIC ACTION OF ETHACRYNIC ACID IN CONGESTIVE HEART FAILURE

BY

DENIS DALEY, M.B., M.R.C.P.

BYRON EVANS, M.D., F.R.C.P.

United Cardiff Hospitals

The introduction of the thiazide group of diuretics was a major advance in the oral treatment of patients with heart failure. Subsequent derivatives and other chemically unrelated compounds have not shown any major therapeutic advantage over the parent drug. Ethacrynic acid (MK-595) is a new diuretic agent. Its diuretic action was first assessed in five patients with congestive heart failure. Its efficacy in an oral dosage of 150 mg. was then compared with 2 ml. of mersalyl given intramuscularly in 10 patients.

Ethacrynic acid is an unsaturated ketone derivative of aryloxyacetic acid. It is different in structure from the thiazides and it does not contain sulphur, nitrogen, or a heavy metal. The first observations of its diuretic action were made in dogs (Schultz *et al.*, 1962; Baer *et al.*, 1962, 1963). Clinical trials in man (Foltz, 1963) showed that ethacrynic acid was a potent oral diuretic with powerful chloruretic and natriuretic effects. Dose-response studies during this early investigation indicated that the maximum response occurred with 200 mg. of the drug, but above this dosage no increase in the diuretic or saluretic effects were noted. Since a sudden and large diuresis is potentially dangerous it was decided, in this present study, to use a dose of 150 mg.

Assessment of Diuretic Action

Methods

Five in-patients with heart failure (Table I) and associated peripheral oedema were studied. They were treated with

bed rest and a diet very low in sodium (25 mEq/24 hours). Fluids were restricted to 800 ml./day and digitalis was given in optimum dosage. The patients were weighed at the same time each day, and when their weights remained steady 24-hour urinary volumes were measured and the 24-hour excretions of sodium, chloride, and potassium were estimated. These observations were continued for three days to establish basal levels. Ethacrynic acid was then given in a single morning dose of 150 mg. orally for five consecutive days and all the above estimations were continued. The serum levels of sodium, chloride, and potassium and the blood urea were measured on the day before ethacrynic acid was started and on the day after the course had been completed. In two patients the duration of the diuretic action was measured. On the first day of treatment the volume of hourly specimens of urine was measured, starting three hours before the drug was given, and this was continued throughout the day.

Results

In all the patients a diuresis occurred during treatment with ethacrynic acid. The amount of the diuresis naturally varied with the degree of peripheral oedema that was present. Thus in three patients (Cases 1, 2, and 3) who had gross pitting oedema of the legs and lower trunk an average of 2,229 ml., 1,840 ml., and 1,896 ml. respectively was excreted daily over the five-day period of treatment. In Cases 4 and 5 there was minimal pitting oedema of