

**The effect of cigarette smoking
on susceptibility to epidemic influenza and on serological
responses to live attenuated and killed subunit
influenza vaccines**

BY J. S. MACKENZIE, I. H. MACKENZIE AND P. G. HOLT

*University Department of Microbiology, Perth Medical Centre, Nedlands
WA 6009*

(Received 9 June 1976)

SUMMARY

The effects of cigarette smoking on the incidence of epidemic influenza and on the serological response to influenza vaccination with killed subunit and live attenuated vaccines have been investigated during comparative vaccine trials in Western Australia. It was found that cigarette smokers with no pre-epidemic haemagglutination-inhibiting (HI) antibody (titres of ≤ 12) were significantly more susceptible to epidemic influenza than non-smokers. Smokers were no more susceptible however, if they had possessed detectable pre-epidemic HI antibody. A significantly higher proportion of smokers sero-converted after receiving the live virus vaccine than their non-smoking counterparts, but this could not be correlated with pre-vaccination HI antibody titres. The longevity of the immune response to the subunit vaccine was severely depressed 50 weeks post-vaccination in smokers who had possessed little or no immunity before vaccination (titres of ≤ 12). This antibody deficit was not observed in live virus vaccinees or subunit vaccinees with pre-vaccination HI antibody (titres of ≥ 24). Post-vaccinal symptoms were similar regardless of vaccine group or smoking history.

INTRODUCTION

An increased susceptibility to epidemic influenza and other respiratory infections has been observed among young adult cigarette smokers when compared with non-smokers (Finklea, Sandifer & Smith, 1969; Finklea *et al.* 1971*a*; Haynes, Krstulovic & Bell, 1966; Parnell, Anderson & Kinnis, 1966). Humoral haemagglutination-inhibiting (HI) antibody titres to influenza were significantly increased among smokers who remained well and minimally increased among smokers who were sick, compared with those of non-smokers, but the persistence of their HI antibody after vaccination was significantly decreased. There was no significant difference, however, between smokers and non-smokers in their immunological response to vaccination with killed influenza vaccine (Finklea *et al.* 1971*b*).

Comparative clinical trials of live attenuated and detergent split influenza virus vaccines have been undertaken in Western Australia (Mackenzie, Mackenzie, Lloyd & Dent, 1975). This report examines the effect of cigarette smoking on

sero-conversion frequencies to live and subunit vaccines, the longevity of the immune response to the two vaccines, and the susceptibility of smoking and non-smoking volunteers to epidemic influenza.

MATERIALS AND METHODS

The volunteers, vaccines, clinical schedules and laboratory techniques have been described in detail previously (Mackenzie *et al.* 1975). In brief, 799 volunteers from two localities in Western Australia (the towns of Collie and Busselton) were randomly assigned to three groups: group A received two doses of a live influenza A virus vaccine administered intra-nasally 2 weeks apart; group B received two doses of a saline control administered by the same route and schedule as group A; and group C received two doses of a killed subunit influenza vaccine administered by deep subcutaneous injection 4 weeks apart. Only male volunteers between the age of 18 and 55, who were in good health and not in influenza high-risk categories, were permitted to receive the live virus vaccine.

The live vaccine, 'Alice' strain, was developed by Recherche et Industrie Thérapeutique, Belgium, as a stable inhibitor-resistant variant of MRC-2, a recombinant isolated by Dr G. C. Schild from a cross between A/England/42/72 (H3N2) and A/PR8/34 (H0N1). This recombinant retained the neuraminidase and haemagglutinin antigens of the A/England/42/72 parent. Each dose contained $10^{7.3}$ egg infectious units in 0.6 ml. The killed subunit vaccine was a commercially available preparation manufactured by the Commonwealth Serum Laboratories, Melbourne. Each dose contained 16,000 haemagglutinating units of A/England/42/72 and 8000 haemagglutinating units of B/Roma/1/67 in 1.0 ml.

Blood samples were collected from volunteers immediately before vaccination to determine pre-vaccination humoral antibody titres, and subsequently before the second dose of vaccine, and at 7, 30 and 50 weeks after vaccination. Serum samples were treated with cholera filtrate to destroy non-specific viral inhibitors, and then titrated in parallel for haemagglutination-inhibiting (HI) antibody. Four haemagglutinating units (HA) of influenza strain MRC-7, which contained the antigenic determinants of A/England/42/72, were incubated with serial twofold serum dilutions overnight at 4°C. before the addition of a 0.5% suspension of fowl erythrocytes. Post-epidemic sera were also tested against 4 HA units of A/Perth/2/73 which was antigenically similar to A/Port Chalmers/1/73.

Volunteers were asked to complete a daily symptoms chart to record any 'influenza-like' symptoms for 7 days after each dose of vaccine. The smoking history and average daily cigarette consumption of volunteers who had smoked for a year or less, who averaged less than one half of a pack of cigarettes per day, or were volunteers who smoked cigars or pipes were excluded from this study.

Table 1. Summary of symptoms recorded after first dose of vaccine by smokers and non-smokers

Group	(A) Live vaccine		(B) Placebo		(C) Subunit vaccine	
	Smokers	Non-smokers	Smokers	Non-smokers	Smokers	Non-smokers
Volunteers recording no symptoms (%)	64	60.5	59.4	59.4	58.6	52.9
Volunteers (%) recording symptoms of:						
Rhinorrhoea	26	25	27.5	26	22.9	25.5
Cough	6.3	6.1	4.4	4.3	10.0	8.5
Sore throat	15.8	16.6	13.1	16.4	15.7	14.4
Headache	12.6	13.9	14.5	17.6	15.7	18.9
Malaise	6.3	7.2	13.0	9.7	11.4	5.9
Joint pains	5.3	5.0	0	3.0	2.9	7.8
Muscle pains	2.1	2.8	4.3	3.6	1.4	3.9
Pain at injection site	0	0	0	0	20.0	21.6
Fever	4.2	5.6	4.3	2.4	8.6	3.3

Table 2. Sero-conversion frequencies between smokers and non-smokers after administration of live virus and subunit vaccines in volunteers with initial HI titres of < 6-12 or 24-96

Smoking history	Vaccine: live virus				
	Pre-vaccination titre	Number of volunteers	Sero-conversion after:		Sero-conversion (%)
			1st dose	2nd dose	
Smokers	< 6-12	82	39	31	85.6
	24-96	29	14	11	
Non-smokers	< 6-12	105	39	40	75
	24-96	43	16	16	
Vaccine: Subunit					
Smokers	< 6-12	65	49	9	87.6
	24-96	24	17	3	
Non-smokers	< 6-12	137	101	15	84
	24-96	50	36	5	

RESULTS

Post-vaccinal symptoms

Symptoms charts were returned by 312 volunteers after receiving their first dose of vaccine. An analysis of the symptoms recorded during the 3 days after vaccination is shown in Table 1 in terms of the type of vaccine and the smoking history of the volunteers. No significant differences were observed between smokers and non-smokers, nor, as has been reported earlier (Mackenzie *et al.* 1975), between the vaccine groups.

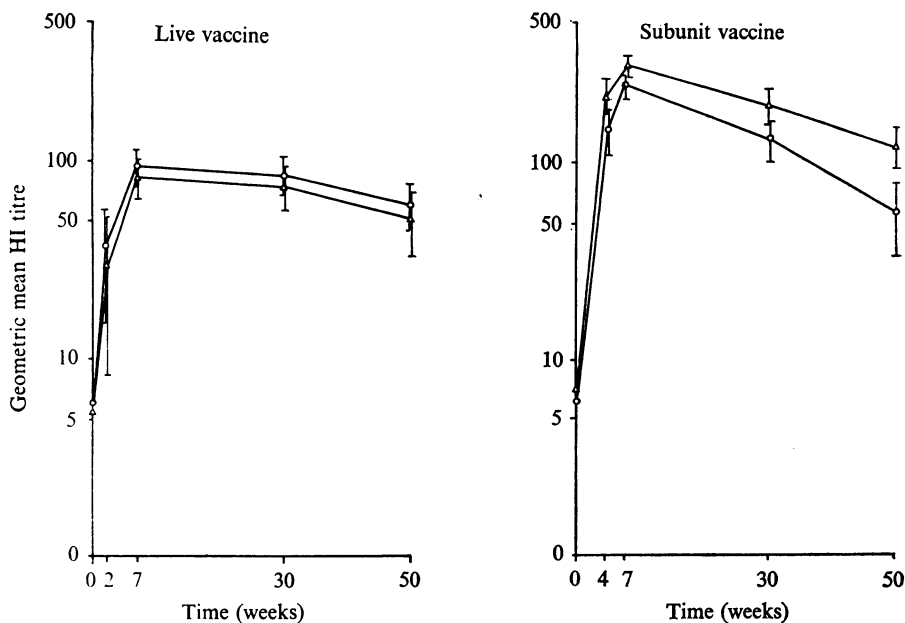


Fig. 1. Longevity of the immune response to live attenuated and killed subunit influenza virus vaccines of smokers and non-smokers with pre-vaccination HI titres of ≤ 12 . O-O, Smokers: Δ - Δ , non-smokers.

Humoral antibody response to vaccination

A fourfold or greater rise in serum HI antibody was assumed to be indicative of sero-conversion. The effect of smoking history on the ability of the vaccines to invoke sero-conversion in volunteers with pre-vaccination HI antibody titres of 96 or less, and the vaccination dose after which sero-conversion occurred, are shown in Table 2. A significantly higher proportion of smokers to non-smokers sero-converted after receiving the live vaccine ($\chi^2(1) = 4.37$, $P = < 0.05$), and a similar trend was observed in the subunit vaccines. There were no significant differences, however, between pre-vaccination HI titres and susceptibility to infection with the live virus vaccine for either smokers or non-smokers. Live virus vaccinees who smoked had a slightly greater chance of sero-converting after the first dose of vaccine.

The longevity of the immune response was determined over a period of 50 weeks after vaccination. The geometric mean HI titres of smokers and non-smokers who had little or no HI antibody before vaccination (HI titres of ≤ 12) are shown in Fig. 1. No difference was observed in the longevity of HI antibody between smokers and non-smokers who had received the live vaccine, but subunit vaccinees who smoked exhibited a significant depression in the longevity of HI antibody by 50 weeks ($t = 2.35$, 111 D.F., $P = < 0.05$). Smoking had no such effect on longevity in volunteers with residual immunity before vaccination (HI titres of ≥ 24).

Table 3. *Effect of smoking on susceptibility to epidemic influenza*

Vaccine group	Pre-epidemic titre	Smoking history	Total no. of volunteers	No. of volunteers with serological evidence of epidemic influenza		
				A/Eng/42/72	A/Perth/2/73	Total
Placebo	< 6-12	Smokers	52	9	10	19 *
		Non-smokers	152	17	15	32 *
Placebo	24-96	Smokers	23	0	0	0
		Non-smokers	79	2	1	3
Live vaccine	24-96	Smokers	59	0	2	2
		Non-smokers	70	1	2	3
Subunit vaccine	24-96	Smokers	35	0	3	3
		Non-smokers	55	2	3	5

* Significant $\chi^2_{(1)} = 4.96$, $P = < 0.05$.

Effect of smoking on susceptibility to epidemic influenza

An influenza epidemic occurred in Western Australia with the majority of cases falling between mid-October and mid-December 1973, approximately 3½-5½ months after vaccination. The epidemic was unusual in that it was much later in the year than normal and two antigenically distinct strains of influenza were isolated. The two strains were antigenically related to A/England/42/72 and A/Port Chalmers/1/73. A fourfold or greater rise in serum HI antibody titres between 7 and 50 weeks after vaccination was construed as evidence of infection with epidemic influenza. All sera were titrated against both strains of virus using MRC-7 (antigenically similar to A/England/42/72) and A/Perth/2/73 (antigenically similar to A/Port Chalmers/1/73).

The incidence of epidemic influenza among smokers and non-smokers in the three vaccine groups is shown in Table 3. Smokers with little or no pre-epidemic HI antibody (HI titres of < 6-12) were significantly more susceptible to epidemic influenza than non-smokers ($\chi^2_{(1)} = 4.96$, $P = < 0.05$), but no such difference was observed between smokers and non-smokers with detectable levels of antibody (HI titres of 24-96), regardless of vaccine history. Thus, in the susceptible population (with no prior immunity), the incidence of epidemic influenza was 36.5% among smokers and 21% among non-smokers.

For logistic reasons, it was not possible to monitor volunteers for clinical influenza during the epidemic period. Instead each subject was provided with a questionnaire, similar to the post-vaccinal symptoms chart, which was used in an attempt to assess the incidence of clinical influenza (Mackenzie *et al.* 1975). No significant information was obtained, however, to correlate clinical symptoms with serological evidence of infection between smokers and non-smokers, or between the two groups of vaccinees.

DISCUSSION

The effect of cigarette smoking on the immune response to influenza vaccination with a killed vaccine and on susceptibility to epidemic influenza have been reported previously (Finklea *et al.* 1969; Finklea *et al.* 1971*b*). It was observed that, before immunization with the killed vaccine, cigarette smokers showed significantly lower titres of HI antibody than non-smokers, but immediately after vaccination this antibody deficit was abolished and the immunological response did not differ significantly between the two groups. However, the HI titres in the smokers had fallen behind their non-smoking counterparts within a few weeks, and by one year after vaccination the titres of humoral HI antibody of the smokers were much lower (Finklea *et al.* 1971*b*). This may have contributed to the increased susceptibility of cigarette smokers to subsequent infection with epidemic influenza (Finklea *et al.* 1969).

The comparative clinical trials of live attenuated and killed subunit influenza virus vaccines carried out in Western Australia (Mackenzie *et al.* 1975) have provided the opportunity to confirm and extend the observations of Finklea and his colleagues. Subjects who had smoked for less than 1 year or who smoked less than one half a pack of cigarettes each day were excluded from this study, as were cigar and pipe smokers.

The susceptibility of cigarette smokers to epidemic influenza was found to be dependent on pre-epidemic titres of HI antibody (Table 3). Only smokers with little or no antibody (titres of ≤ 12) were significantly more susceptible than their non-smoking counterparts. No difference in susceptibility could be demonstrated if smokers had prior immunity (titres of ≥ 24), whether from vaccination or previous natural infection. In the study reported by Finklea *et al.* (1969), the epidemic was caused by a novel influenza virus strain, A/Hong Kong/68, which had undergone a partial antigenic shift. Their subjects, therefore, would also have had little or no prior immunity. Post-epidemic HI titres showed little difference between smokers and non-smokers who were clinically ill, but the titres of smokers who remained clinically well were significantly higher, suggesting that smokers contracted more subclinical influenza. It was not possible to monitor the volunteers clinically during the epidemic period in the present study, so the increased susceptibility was determined from serological evidence only.

Post-vaccinal sero-conversion frequencies to the live attenuated and killed subunit vaccines were analysed in terms of the smoking history of the volunteers (Table 2). A significantly higher proportion of smokers were found to sero-convert after receiving the live virus vaccine, and a similar but minimal trend was observed among subunit vaccinees. Thus, like the epidemic data, cigarette smokers were more susceptible to the live virus. Moreover, there was a slightly greater chance for the smokers to sero-convert to the first dose of live vaccine. Unlike the epidemic data, however, there was no significant correlation between susceptibility and pre-vaccination HI antibody levels. The reasons for this are unknown, but must reflect to some degree the amount of virus present in the vaccine and its virulence.

No significant differences were found in the height of the immune response

between smokers and non-smokers with either vaccine up to 45 days after vaccination, regardless of their pre-vaccination antibody status. The persistence of HI antibody, however, was severely depressed in smokers who had received subunit vaccine and who had possessed no detectable antibody before receiving the vaccine (Fig. 1). This antibody deficit was apparent at 30 weeks, but not significant until 50 weeks, after vaccination. No such effect was observed among smokers who had received the live virus vaccine, or among smokers with residual immunity who had received the subunit vaccine. The longevity of the immune response among smokers to a killed vaccine was also shown to be depressed 1 year after vaccination by Finklea and colleagues (Finklea *et al.* 1971*b*), but they did not attempt a correlation with residual pre-vaccination immunity. They further reported that pre-vaccination geometric mean HI titres were lower for smokers than non-smokers, but their finding could not be confirmed in this study (Table 2). It is interesting to note, however, that killed vaccines before 1968 were whole virus vaccines rather than the subunit vaccines currently in use.

Despite many reports of an increased incidence of upper respiratory disease in smokers, there was no difference in symptoms recorded by smokers and non-smokers after receiving the live virus vaccine, nor between the different vaccine groups (Table 1). It should be remembered, however, that the assessment of self-diagnosed symptoms charts or questionnaires has a number of inherent problems (Mackenzie & Houghton, 1974), not least the correlation of clinical symptoms with serological evidence.

Cigarette smoking has been shown to have profound effects on body defence mechanisms in man and in laboratory animal models, and cigarette-smoke-induced changes in immunological function has been suggested as an important aetiological factor in a number of the respiratory diseases associated with this habit (Holt, Thomas & Keast, 1974). In a murine model system chronic cigarette smoke inhalation has been found to lead to a severe depression in humoral immunity and in cell-mediated immunity, both within the respiratory tract and distal to it (Thomas, Holt & Keast, 1973*a, b*, 1974; Chalmer, Holt & Keast, 1975). The primary immune response to intratracheal stimulation with sheep erythrocytes was depressed and the secondary response deleted, which would suggest that the capacity to mount an immune response to respiratory infections could be seriously impaired. Such an effect has been observed. Prolonged exposure of mice to cigarette smoke depressed the humoral immune response following infection with live influenza virus, and decreased the frequency of sero-conversion. Conversely, short-term exposure of mice to cigarette smoke resulted in an enhancement of the immune response (Mackenzie, 1976). Furthermore, exposure of influenza-infected mice to cigarette smoke increased the mortality frequency (Spurgash, Ehrlich & Petzold, 1968). The depressed humoral immune response on prolonged exposure of mice was not observed in the volunteers in the present study, possibly because the mice had had no previous exposure to influenza and therefore lacked original antigenic stimulation (original antigenic sin).

Cell-mediated immunity in smoking mice was also profoundly affected by chronic exposure to cigarette smoke (Thomas, Holt & Keast, 1973*b*; Chalmer,

Holt & Keast, 1975). The importance of local and systemic cell-mediated immunity in influenza infections has not been determined, although various cell-mediated immune responses have been described following infections with influenza in a murine system (Cambridge, Mackenzie & Keast, 1976).

In man, cigarette smoking has been shown to lead to significant amounts of C-reactive protein in the serum, an abnormal protein associated with inflammatory or invasive neoplastic diseases (Heiskell, Miller, Aldrich & Carpenter, 1962). Titres of non-specific agglutinins were depressed in smokers' sera relative to non-smokers (Fletcher, Sumney, Langkamp & Platt, 1969), together with depressed level of humoral IgG (Vos-Brat & Rümke, 1969). Nymand (1974) observed that pregnant women who smoked had a significantly lower incidence of lymphocytotoxins in their sera, and were more prone to infections of the urinary tract, and to febrile and non-febrile virus diseases.

Lavage fluids from smokers consistently yielded more pulmonary alveolar macrophages than those from non-smokers, which exhibited elevated metabolic rates (Harris, Swenson & Johnson, 1970), increased activity of lysosomal hydrolases (Martin, 1973), and a failure to respond to migration inhibitory factor (Warr & Martin, 1973). Smokers have also been shown to invariably exhibit leukocytosis characterized by increased numbers of peripheral leukocytes (Friedman *et al.* 1973; Silverman, Potvin, Alexander & Chretien, 1975), including thymus-dependent lymphocytes (Silverman *et al.* 1975).

Two recent reports also suggest that systemic cellular immune function in human smokers may exhibit biphasic changes, analogous to those observed in smoking mice (Thomas *et al.* 1973*b*; Chalmer *et al.* 1975). Silverman *et al.* (1975) reported that smokers under the age of 40 exhibited elevated phytohaemagglutinin responses, whereas older heavy smokers have been shown to exhibit significantly depressed phytohaemagglutinin reactivity (Vos-Brat & Rümke, 1969).

Cigarette smoking may thus affect many aspects of the host immune system which may reflect on the persistence of humoral HI antibody and the susceptibility to epidemic influenza described in this study. Additional investigations are needed to assess the protective capacity of vaccines in smokers and others subjected to gaseous pollutants, to ascertain the mechanisms involved in immune depression, and to study the neonatal hazard that is likely to exist to children of mothers who smoke.

This study was supported by grants from Smith Kline & French Laboratories (Australia) Limited, The Australian Tobacco Research Foundation and the National Health and Medical Research Council.

REFERENCES

- CAMBRIDGE, G., MACKENZIE, J. S. & KEAST, D. (1976). Cell-mediated immune response to influenza virus infection in mice. *Infection and Immunity* **13**, 36-43.
- CHALMER, J., HOLT, P. G. & KEAST, D. (1975). Cell-mediated immune response to transplanted tumors in mice chronically exposed to fresh cigarette smoke. *Journal of the National Cancer Institute* **52**, 1129-34.
- FINKLEA, J. F., HASSELBLAD, V., SANDIFER, S. H., HAMMER, D. I. & LOWRIMORE, G. R. (1971a). Cigarette smoking and acute non-influenzal respiratory disease in college males. *American Journal of Epidemiology* **93**, 457-62.
- FINKLEA, J. F., SANDIFER, S. H. & SMITH, D. D. (1969). Cigarette smoking and epidemic influenza. *American Journal of Epidemiology* **90**, 390-9.
- FINKLEA, J. F., HASSELBLAD, V., RIGGAN, W. B., NELSON, W. C., HAMMER, D. I. & NEWILL, V. A. (1971b). Cigarette smoking and haemagglutination inhibition response to influenza after natural disease and immunization. *American Review of Respiratory Disease* **104**, 368-76.
- FLETCHER, R. D., SUMNEY, D. L., LANGKAMP, H. H. & PLATT, D. (1969). The ability of human serum to agglutinate sheep erythrocytes and the effect of tobacco mosaic virus. *American Review of Respiratory Disease* **100**, 92-4.
- FRIEDMAN, D. G., SIEGELAUB, A. B., SELTZER, C. C., FELDMAN, R. & COLLEN, M. F. (1973). Smoking habits and the leukocyte count. *Archives of Environmental Health* **26**, 137-43.
- HARRIS, J. O., SWENSON, E. W. & JOHNSON, J. E. (1970). Human alveolar macrophages: comparison of phagocytic ability, glucose utilization, and ultrastructure in smokers and non-smokers. *The Journal of Clinical Investigation* **49**, 2086-96.
- HAYNES, W. F., KRSTULOVIC, V. J. & BELL, A. L. L. (1966). Smoking habit and incidence of respiratory tract infections in a group of adolescent males. *American Review of Respiratory Diseases* **93**, 730-5.
- HEISKELL, C. L., MILLER, J. N., ALDRICH, J. J. & CARPENTER, C. M. (1962). Smoking and serologic abnormalities. *Journal of the American Medical Association*, **181**, 647-7.
- HOLT, P. G., THOMAS, W. R. & KEAST, D. (1974). Smoking and immunity. *Lancet* **i**, 152.
- MACKENZIE, J. S. (1976). The effect of cigarette smoke on influenza virus infection: a murine model system. *Life Sciences*, August 1976 (in press).
- MACKENZIE, J. S. & HOUGHTON, M. (1974). Influenza infections during pregnancy: association with congenital malformations and with subsequent neoplasms in children, and potential hazards of live virus vaccines. *Bacteriological Reviews* **38**, 356-70.
- MACKENZIE, J. S., MACKENZIE, I. H., LLOYD, J. & DENT, V. (1975). Comparative trials of live attenuated and detergent split influenza virus vaccines. *Journal of Hygiene* **75**, 425-43.
- MARTIN, R. R. (1973). Altered morphology and increased acid hydrolase content of pulmonary macrophages from cigarette smokers. *American Review of Respiratory Disease* **107**, 596-601.
- NYMAND, G. (1974). Maternal smoking and immunity. *Lancet* **ii**, 1379-80.
- PARNELL, J. L., ANDERSON, D. O. & KINNIS, C. (1966). Cigarette smoking and respiratory infections in a class of student nurses. *New England Journal of Medicine* **274**, 979-84.
- SILVERMAN, N. A., POTVIN, C., ALEXANDER, J. C. & CHRETIEN, P. B. (1975). *In vitro* lymphocyte reactivity and T-cell levels in chronic cigarette smokers. *Clinical and Experimental Immunology* **22**, 285-92.
- SPURGASH, A., EHRLICH, R. & PETZOLD, R. (1968). Effect of cigarette smoking on resistance to respiratory infection. *Archives of Environmental Health* **16**, 385-91.
- THOMAS, W. R., HOLT, P. G. & KEAST, D. (1973a). Effect of cigarette smoking on primary and secondary humoral responses in mice. *Nature, London* **243**, 240-1.
- THOMAS, W. R., HOLT, P. G. & KEAST, D. (1973b). Cellular immunity in mice chronically exposed to fresh cigarette smoke. *Archives of Environmental Health* **27**, 372-5.
- THOMAS, W. R., HOLT, P. G. & KEAST, D. (1974). The development of alterations in the primary immune response of mice by exposure to fresh cigarette smoke. *International Archives of Allergy and Applied Immunology* **46**, 481-6.
- WARR, G. A. & MARTIN, R. R. (1973). *In vitro* migration of human alveolar macrophages: effects of cigarette smoking. *Infection and Immunity* **8**, 222-7.
- VOS-BRAT, L. C. & RÜMKE, P. (1969). Immunglobuline concentraties, PHA reacties van lymfocyten *in vitro* en enkele antistof titers van gezonde rokers. *Jaarboek van Kankeronderzoek en Kankerbestrijding in Nederland* **19**, 49-53.