I thank Prof. M. Wada for his valuable advice in preparing this report. I also thank Hoffmann-La Roche Inc. for the supply of acetylthiocholine and butyrylthiocholine, and Merck and Co. Inc. for DFP.

Tsuyoshi Aoki

Physiological Laboratory,
Tohoku University School of Medicine,
Sendai, Japan.

[1] Hurley, H. J., and Mescon, H., *Proc. Soc. Exp. Biol. Med.*, **92**, 103 (1956).
[2] Hurley, H. J., Shelley, W. B., and Koelle, G. B., *J. Invest. Dermat.*, **21**, 139 (1953).
[3] Hellmann, K., *Nature*, **169**, 113 (1952).
[4] Hellmann, K., *J. Physiol.*, **129**, 454 (1955).
[5] Hashimoto, K., Ogawa, K., and Lever, W.F., *J. Invest. Dermat.*, **40**, 15 (1963).
[6] Winkelmann, R. K., and Schmit, R. W., *J. Invest. Dermat.*, **33**, 185 (1959).
[7] Takahashi, Y. (to be published).
[8] Koelle, G. B., and Friedenwald, J. S., *Proc. Soc. Exp. Biol. Med.*, **70**, 617 (1949).
[9] Gomori, G., *Microscopic Histochemistry: Principles and Practice*, 211 (Univ. Chicago Press, 1952).
[10] Montagna, W., *The Structure and Function of Skin*, 354 (Academic Press, New York and London, 1962).

PATHOLOGY

Induction of Pulmonary Tumours in Mice by Nitrosonornicotine, a Possible Constituent of Tobacco Smoke

Nitrosamines could be formed in cigarette smoke by reactions between secondary amines and oxides of nitrogen[1,2]. Several secondary amines, including anabasine, nornicotine, dimethylamine, diethylamine, pyrrolidine, piperidine and proline, have been detected in cigarette smoke, which also contains about 200 parts per million of oxides of nitrogen (as the sum of NO and NO_2) (refs. 3 and 4). The nitrosamine derivatives of some of these amines have already been shown to be carcinogenic[5,6]. We have reported that nitrosoanabasine administered in the drinking water induced benign and malignant tumours of the œsophagus of rats[1]. In parallel with these biological investigations attempts have been made to detect nitrosoanabasine and nitrosonornicotine in cigarette smoke. So far these attempts have not been successful, although methods capable of detecting small amounts (for example, 5 μg) of specific nitrosamines have been developed[1]. Lack of success may be due either to instability of nitrosamines under the conditions prevailing in cigarette smoke or to interference in their detection by other constituents of the smoke. Nitrosoanabasine could not be detected in cigarette smoke condensates to which it had been deliberately added nor in the smoke of cigarettes which had been impregnated with the substance before smoking. If these negative results are due to instability of the nitrosamines, the possibility of their biological importance is only ruled out if their half-lives are very short. Further investigations into these problems are in progress.

Of the secondary amines in tobacco, nornicotine is probably the most abundant. The smoke from a single cigarette contains about 5 mg nicotine and 100 μg of secondary amine alkaloids, including anabasine, myosmine and nornicotine[7]. Nitrosonornicotine was prepared by treating nornicotine (Fluka, Buchs, Switzerland) with sodium nitrite in dilute hydrochloric acid solution. The nornicotine formed was extracted from the reaction mixture with ether. The ether extract was dried, the ether removed by distillation and the residual nitrosonornicotine distilled under reduced pressure (b.p. 190°–192° at 0·5 mm).

The nitrosonornicotine was tested for carcinogenicity in mice. Twenty male and 20 female mice of the Chester Beatty stock strain were injected once-weekly intraperitoneally with 0·1 ml. 2 per cent nitrosonornicotine dissolved in arachis oil for 41 weeks. Fifteen male and 15 female mice were injected each week with arachis oil only as controls. Mice for the experiment were randomized between the test and control groups at the start of the experiment. The first injection was given when the mice were approximately six weeks old.

In the test group 14 males and 11 females died during the first 7 months of treatment. Twenty of these 25 animals were examined at autopsy and none had tumours. However, of 8 animals dying since the eighth month, 7 (5 females and 2 males) which were autopsied had multiple pulmonary adenomas, the nature of which was confirmed histologically. Several of these tumours showed local invasion of lung and into bronchi, but none had metastasized to lymph glands or to sites outside the thoracic cavity. In 5 out of the 7 tumour-bearing animals the pulmonary lesions exceeded 30 in number and were too numerous to count with accuracy. In addition to the pulmonary tumours one of the males had a localized lymphosarcoma arising in one kidney. The experiment is now in its eleventh month and 7 animals are still under observation. Of the control group 12 males and 12 females died without tumours at any site before the eighth month. The remaining 6 animals were killed during the eleventh month; one of these had a solitary small pulmonary adenoma, but none of the others had any tumours.

These results indicate that, in the doses given, nitrosonornicotine is a potent carcinogen for mouse-lung. The significance of this in relation to the human smoking habit remains to be elucidated.

We thank Mrs. J. Merryweather for skilled technical assistance. This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service, and the Tobacco Research Council.

E. Boyland
F. J. C. Roe
J. W. Gorrod

Chester Beatty Research Institute,
Institute of Cancer Research,
Royal Cancer Hospital,
Fulham Road,
London, S.W.3.

[1] Boyland, E., Roe, F. J. C., Gorrod, J. W., and Mitchley, B. C. V., *Brit. J. Cancer* (in the press).
[2] Druckrey, H., and Preussmann, R., *Naturwissen.*, **49**, 498 (1962).
[3] Haagen-Smit, A. J., Brunelle, M. F., and Harø, J., *Arch. Indust. Health*, **20**, 399 (1959).
[4] Bokoven, C., and Niessen, H. J., *Nature*, **192**, 458 (1961).
[5] Magee, P. N., and Barnes, J. M., *Brit. J. Cancer*, **10**, 114 (1956).
[6] Druckrey, H., Steinhoff, D., Beather, H., Schneider, H., and Klärner, P., *Arzneimittel-Forsch.*, **13**, 320 (1963).
[7] Quin, L. D., *J. Org. Chem.*, **24**, 914 (1959).

Prevention of Tumour Induction in SV_{40}-infected Hamsters

Several investigators have attempted to prevent induction of tumours in animals infected by oncogenic viruses using immunization procedures during the latent period, that is, before the appearance of visible (palpable) tumours. In these experiments, virus-infected animals were inoculated during the latent period by live or killed tumour cells or by their components. Zilber and Baidakova[1] indicated that the Bittner mammary tumour development in immunized mice could not be prevented although the duration of the latent period was longer. Goldner *et al.*[2] failed to immunize SV_{40}-infected hamsters by 'Björklund-type' tumour antigen; some stimulation effect on the tumour growth in such animals was obtained.

It was shown that adult animals inoculated by an oncogenic virus become resistant to the transplantation of tumours induced by the same virus[3,4].