BOYLAN1965

Institute of Morbid Anatomy

Division of Cancer Research

INTERNATIONAL CONFERENCE

ON

LUNG TUMOURS IN ANIMALS

PERUGIA, 1965

24th to 29th JUNE

Monday, June 28th, 1965

Session XIV. 9.00 to 9.45

Chairman . . . J. BALÒ

9.00 to 9.45 Conference Lecture :
R. KINOSITA (U.S.A.) : *Experimental lung tumors in animals.*

9.45 to 10.00 Break

Meeting 6B. **Experimental lung tumours by chemicals**

Session XV. 10.00 to 11.00

Chairman . . . E. BOYLAND

10.00 to 10.15 J. BALO' (Hungary) : *Lung tumours in laboratory animals indicating the tumour-provoking property of pharmaca.*

10.15 to 10.20 Discussion.

10.20 to 10.35 R. SCHOENTAL (U.K.) : *The induction of lung tumours in mice and rats by diazomethane and by N-alkyl-N-nitrosourethanes.*

10.35 to 10.40 Discussion.

10.40 to 10.55 P.N. MAGEE (U.K.) : *Alkylation of nucleic acids in the lung and other organs by carcinogenic nitroso compounds.*

10.55 to 11.00 Discussion.

11.00 to 11.20 Coffee

Session XVI. 11.20 to 13.00

Chairman . . . D.B. CLAYSON

11.20 to 11.35 E. BOYLAND and F.J.C. ROE (U.K.) : *Carcinogenic nitrosamines which may be present in cigarette smoke.*

11.35 to 11.40 Discussion.

11.40 to 11.55 S. TAKAYAMA and K. OOTA (Japan) : *Induction of malignant lung tumours in various strains of mice by oral administration of N-nitrosodimethylamine and N-nitrosodiethylamine.*

11.55 to 12.00 Discussion.

12.00 to 12.15 C. HOCH-LIGETI (U.S.A.) : *Enzyme and protein studies in the lung of rats during pulmonary carcinogenesis by nitrosamine derivative.*

12.15 to 12.20 Discussion.

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Lung Tumours in Animals
Internat Conf. on Lung Tumours in
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CARCINOGENIC NITROSAMINES WHICH MAY BE PRESENT IN CIGARETTE SMOKE

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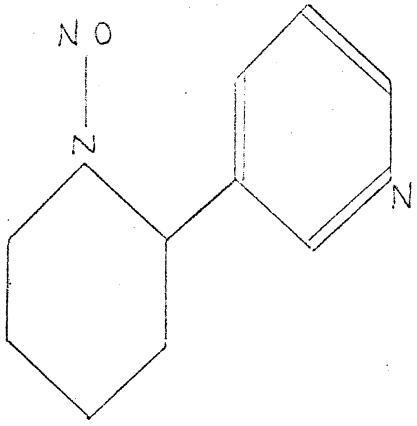
We would like to thank Mr. J. Gorrod for synthesising the compounds used in these experiments. This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, the Tobacco Research Council, and by the Public Health Service Research Grant No. CA-03188-08 from the National Cancer Institute, U.S. Public Health Service.

INTRODUCTION

Oxides of nitrogen are present in tobacco smoke (Haagen-Smit, Brunéle and Hara, 1959; Bokhoven and Niessen, 1961). Similarly, the presence of secondary amines such as nornicotine and anabasine (Quinn, 1959) has been demonstrated. Particularly in the more acid conditions of cigarette smoke as opposed to cigar or pipe smoke, the oxides of nitrogen are likely to react with amines to form nitrosamines. Thus nitrosamines such as nitrosoanabasine(I) and nitrosonornicotine(II) may be formed. These compounds are closely related to carcinogenic nitrosamines such as nitrosopiperidine(III) and nitrosomethylaniline(IV). Indeed nitrosoanabsine is N-nitroso-2-(2' pyridyl) piperidine. Nitrosopiperidine and nitrosomethylaniline are amongst the wide variety of nitrosamines shown to be carcinogenic by Druckrey, Preussmann, Schmahl and Müller(1961, 1962).

The work presented here is of two kinds: firstly, tests of nitrosoanabasine and nitrosonornicotine for carcinogenicity in laboratory animals, and secondly, attempts to detect these two substances in tobacco smoke. Details of part of the work have been published elsewhere (Boyland, Roe, Gorrod and Mitchley, 1964; Boyland, Roe and Gorrod, 1964), however, the induction of liver as well as lung tumours in mice by nitrosonornicotine has not previously been reported.



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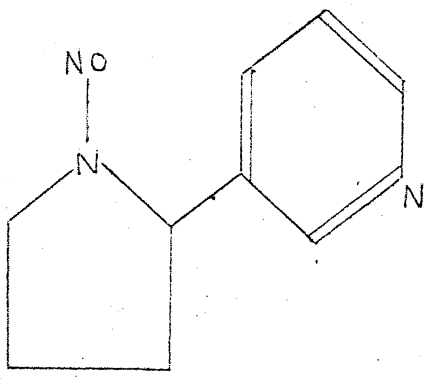

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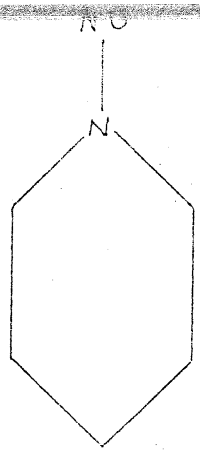

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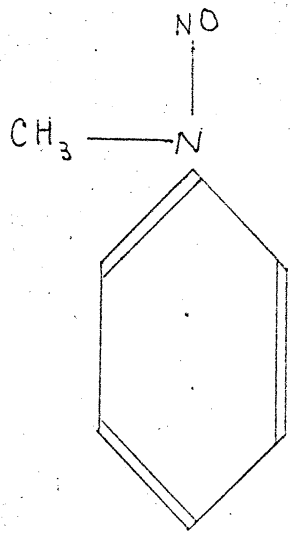

IV

INDUCTION OF OESOPHAGEAL TUMOURS IN RATS BY NITROSOANABASINE

Nitrosoanabasine, a viscous oil, was synthesised by treatment of anabasine with sodium nitrite in dilute hydrochloric acid solution and purified by distillation under reduced pressure. Sixteen male and sixteen female albino rats of the Chester Beatty strain were given nitrosoanabasine at a concentration of 0.2 per cent in the drinking water on six days of each week from the age of 7 weeks. Treatment was continued throughout life. The rats were housed eight animals of the same sex per cage and were examined regularly. Sick animals were killed and subjected to thorough post mortem examination.

Groups of 16 male and 16 female rats were similarly treated with (a) nitrosopiperidine, (b) nitrosoanabasine, (c) left untreated as controls. Rats of each sex were randomized between the four treatment groups at the beginning of the experiment.

Thirteen out of the 16 males and 12 of the 16 females treated with nitrosoanabasine developed squamous tumours of the oesophagus. In four of the males and one of the females malignant tumours were present. Animals with large and multiple oesophageal tumours lost weight and became sick. Otherwise, the presence of oesophageal tumours was not recognised until autopsy was undertaken. Two animals which died before the end of the 10th month of the experiment had no oesophageal tumours. Only 3 out of 13 rats which died between the 10th and 15th month had no tumour of the oesophagus, and none which died after 15 months was without such tumours.

By comparison, no oesophageal tumours were seen in the untreated controls. All but two of the rats treated with nitrosomethylaniline also developed oesophageal tumours. In this case the induction period

appeared to be shorter and the incidence of malignant lesions higher. Thus, 11 cases of malignant oesophageal tumours had been seen in the nitrosomethylaniline treated rats before the first malignant tumour attributable to nitrosoanabasine was seen. Altogether, 12 of the 16 males and 8 of the 16 females treated with nitrosomethylaniline developed malignant oesophageal tumours.

The carcinogenic activity of nitrosopiperidine was even more striking. Between the 5th and 10th month of treatment all the animals died, 26 with oesophageal tumours (16 malignant) and 23 with liver tumours (10 malignant). Only 2 rats, one of each sex, died without either a liver tumour or an oesophageal tumour.

A curious feature with all three compounds was the almost complete absence of lesions in the forestomach epithelium. Even in animals with multiple oesophageal tumours the forestomach epithelium appeared more or less normal. Isolated papillomas were seen in the forestomach of 4 rats and multiple papillomas in 1 rat of the group treated with nitrosomethylaniline. Otherwise, apart from slight epithelial hyperplasia, no changes were observed.

Figures 1 and 2 illustrate some of the oesophageal tumours and Table I shows the incidence of neoplasms. With the exception of the adenocarcinoma of the salivary gland the tumours shown in the final column of Table I are not attributable to treatment, since such tumours arise fairly commonly in untreated rats of the same strain.

It was concluded from these results that nitrosoanabasine administered in the drinking water is carcinogenic for the oesophagus of the rat, but less potent in this respect than nitrosopiperidine or nitrosomethylaniline.

INDUCTION OF TUMOURS OF LUNG AND LIVER IN MICE BY NITROSONORNICOTINE

Nitrosonornicotine, like nitrosoanabasine, is a viscous oil. It was prepared by treating nornicotine with sodium nitrite in dilute hydrochloric acid solution, and purified by distillation under reduced pressure.

Twenty male and 20 female six-week old mice of the Chester Beatty Stock strain were injected intraperitoneally with 0.1 ml. 2 per cent nitrosonornicotine dissolved in arachis oil. Injections were repeated once-weekly for 41 weeks. Fifteen comparable mice of each sex were injected with arachis oil only as a control. Mice of each sex were randomized between test and control groups at the start of the experiment. 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. Of 6 males and 9 females which died or were killed between the 7th and 12th months of the experiment, all, with the possible exception of one which could not be examined post mortem because of decomposition, had multiple pulmonary tumours. Some of the tumours were malignant, as judged by invasion of surrounding lung tissue and intrapulmonary metastasis (see Fig. 3.). No extra pulmonary metastases were seen. In addition, 4 of the males and 2 of the females had benign or malignant parenchymal-cell tumours of the liver. 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.

It was concluded that nitrosonornicotine is carcinogenic both for the lung and for the liver of mice.

DETECTION OF NITROSAMINES IN TOBACCO SMOKE

Attempts have been made to detect nitrosoanabasine and nitrosonornicotine in tobacco smoke. Both can be detected on either paper chromatograms or on thin-layer chromatograms. They can be reduced with zinc and acetic acid to hydrazine derivatives, which give colours with p-dimethylaminocinnamaldehyde. These nitrosamines also react with an acidic solution of 2-(N-benzylanilinemethyl)imidazoline (Antistin) to give yellow colours after 20 minutes which gradually change to green-blue during 24 hours.

A more sensitive test in which the nitrosamines are decomposed in the presence of p-chloraniline-producing a diazonium compound which is then coupled with N-(1-naphthyl)ethylenediamine - has also been developed.

All these methods have been used in attempts to detect these substances in tobacco smoke, but so far without success. Other constituents of the smoke appear to interfere with the reactions, for even when nitrosoanabasine was injected into cigarettes before smoking, none was detected in the smoke. Moreover, nitrosoanabasine added to smoke condensate cannot be detected by these methods. Attempts to overcome these difficulties in detecting small amounts of nitrosoanabasine and other nitrosamines in cigarette smoke are being continued in the light of the positive results obtained in the biological experiments.

DISCUSSION

Nitrosoanabasine and nitrosonornictone are but two examples of nitrosamines which could, theoretically, be produced in cigarette smoke during pyrolysis. Many other amines have been detected in tobacco smoke including dimethylamine, diethylamine, pyrollidine, piperidine and proline. The nitroso derivatives of all of these except proline have been shown to be carcinogenic by Druckrey et al (1962). In view of the potency of this class of carcinogen it is important to ascertain as soon as possible whether substances of this type are indeed present in tobacco smoke. Neurath, Pirmann and Wichern (1964) have recently described an analytical method involving the transformation of N-nitroso-compounds into more stable 5-nitro-2-hydroxy-benzal derivatives. It will be interesting to see whether nitrosamines can be detected in tobacco smoke by this method.

It cannot be assumed that the whole of the carcinogenicity of tobacco smoke could be explained by the production of nitrosamines during pyrolysis. Unburnt tobacco is manifestly carcinogenic both for man and experimental animals (see Roe and Walters, 1965, for review), whilst both experimental evidence and evidence from epidemiological studies on ex-smokers suggest that constituents with tumour-promoting rather than fully carcinogenic activity may play a significant role (Roe, Salaman, Cohen and Burgan, 1959; Roe, 1962; Doll and Hill, 1964).

SUMMARY

1. Nitrosamines such as nitrosoanabasine and nitrosonornicotine may be formed in tobacco smoke by a reaction between oxides of nitrogen and secondary amines.

2. Nitrosoanabasine has induced benign and malignant oesophageal tumours when administered to rats over a prolonged period in the drinking water.

3. Nitrosonornicotine induced multiple pulmonary adenomas and multiple hepatomas in mice when administered by repeated intraperitoneal injection in arachis oil.

4. Attempts to detect either of these nitrosamines in cigarette smoke have been unsuccessful, possibly because of interference by other constituents of the smoke.

Legends

Fig. 1. Bisected stomach and oesophagus from rat given 0.2 per cent nitrosopiperidine in drinking water for six months. Multiple papillomata are present in the oesophagus.

Fig. 2. Squamous carcinoma invading the muscular wall of the oesophagus from a rat given 0.2 per cent nitrosoanabasine in the drinking water for 14 months. H. and E. X255.

Fig. 3. Lung from mouse given 41 intraperitoneal injections of 0.1 ml 2 per cent nitrosonornicotine in arachis oil. This animal died after 12 months and had multiple adenomatous tumours of the lung. The large tumour shown here is extending into bronchi and there are several small metastases from it, together with a second primary adenoma in the remainder of the lung. H. and E. X8

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Carcinogenic Nitrosamines which may be present in cigarette smoke.

E. Boyland and F.J.C. Roe

Many nitrosamines are carcinogenic at different sites in animals. As nitrosamines are formed by the reaction of nitrogen oxides with secondary amines, both of which are present in cigarette smoke, nitrosamines may occur in cigarette smoke. Anabasine and nornicotine are tobacco alkaloids which are secondary amines, converted by nitrosation to nitrosoanabasine and nitrosonornicotine. Nitrosoanabasine, which can be considered as a derivative of the carcinogenic nitrosopiperidine, induced cancer of the oesophagus when administered to rats in drinking water (Boyland, Roe, Gorrod and Mitchley; British Journal of Cancer, 1964, 18, 265). Nitrosonornicotine, on injection into mice, induced pulmonary tumours, some of which invaded the lungs and bronchi (Boyland, Roe and Gorrod; Nature, 1964, 202, 1126). Nitrosonornicotine also induced liver tumours in mice. Nitrosamines of this type might therefore be the active lung carcinogens of cigarette smoke. Attempts to detect these nitrosamines in cigarette smoke in this laboratory have been unsuccessful, but the compounds are reactive and disappear on mixing with cigarette tar.

Fig. 1

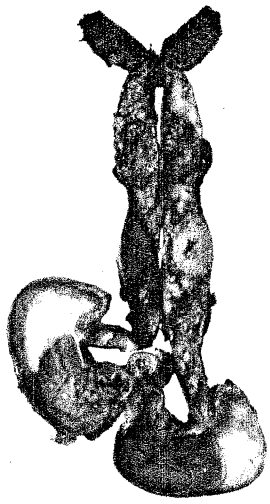
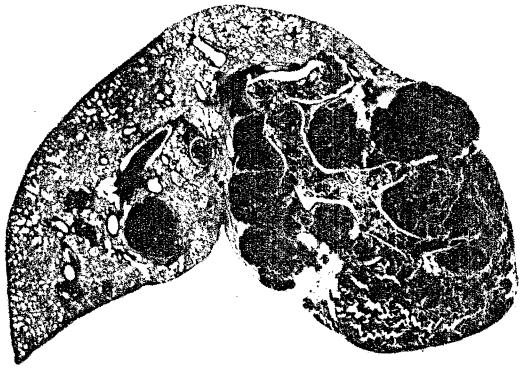


Fig. 3



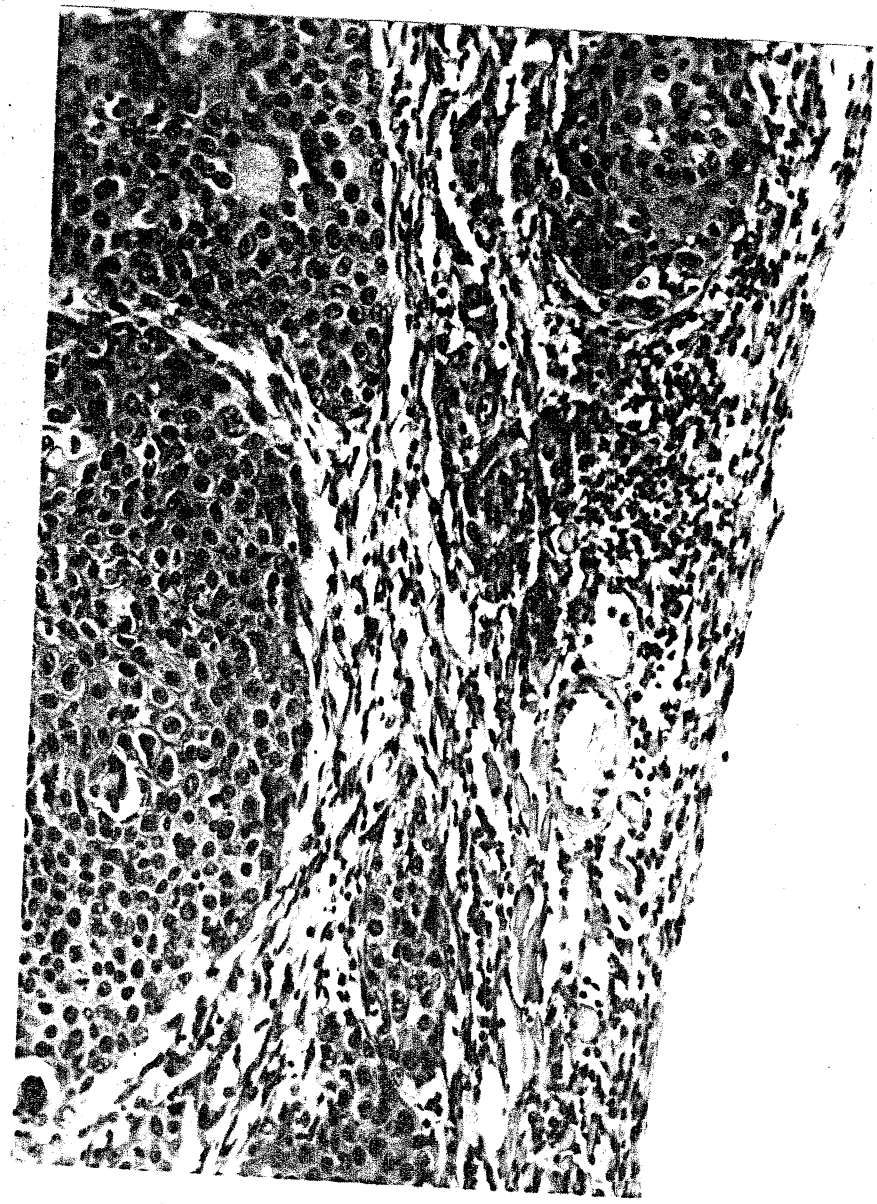


Fig. 2