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THE ROLE OF 3,4-BENZOPYRENE IN EXPERIMENTAL TOBACCO
CARCINOGENESIS

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It is well established that tumours may develop in mouse skin in response to repeated application of the condensate obtained from cigarettes smoked artificially in smoking machines. For this result to be obtained, it is necessary to apply the condensate in large doses over a long period.

In a previous paper (1) my colleagues and I pointed out that the concentration of those constituents of cigarette smoke condensate known to be carcinogenic was inadequate to account for the observed carcinogenicity of the condensate as a whole. For example, it was calculated that the concentration of benzopyrene is less than 2% of that necessary to account for the carcinogenicity of the condensate.

The studies of BERENBLUM and SHUBIK (2) and later of SALAMAN and myself (3) indicated that subcarcinogenic doses of carcinogens *initiated* a neoplastic change which was only realised in the form of a tumour if a *promoting* agent or *co-carcinogen* was subsequently, or simultaneously, applied.

In 1959 we reported that the phenolic fraction of cigarette smoke condensate possessed marked co-carcinogenic activity (1). Mice treated once with a subcarcinogenic dose of 9,10-dimethyl-1,2-benzanthracene (DMBA) and then repeatedly with the phenolic fraction of smoke condensate developed benign and malignant skin tumours. Mice treated with either agent alone developed no tumours. In the light of this discovery, it was postulated that the carcinogenic activity of smoke condensate is due to enhancement of the effects of the low concentrations of carcinogens, such as benzopyrene, by co-carcinogenic phenolic constituents. The fact that it is possible to enhance the carcinogenic activity of smoke condensates by simultaneous or prior treatment with initiating agents but not by simultaneous or subsequent treatment with promoting agents, has led to the view (1) that the tumour promoting potentiality of smoke condensate is in excess of its tumour initiating potentiality. Despite growing evidence in favour of this explanation of the carcinogenic activity of smoke condensate (4), the attention of chemists has tended to centre

on the content of 3,4-benzopyrene, as if the carcinogenic effect of this constituent could, by itself, account for the carcinogenicity of smoke condensate.

The purpose of the experiment to be described, was to assess the role of benzopyrene by comparing the carcinogenicity of samples of unadulterated cigarette smoke condensate with that of samples of condensate to which benzopyrene had been *added* in three different measured amounts. For this purpose large amounts of smoke condensate were kindly supplied by Dr. F. G. Bock, of the Roswell Park Memorial Institute, Buffalo, N.Y., U.S.A. Freshly obtained condensate was flown from Buffalo at 6 week intervals packed in solid carbon dioxide. After arrival, it was stored in a refrigerator at 4°C until used. Dr. Lindsey, of the Sir John Cass Institute in London, kindly made an analysis of a typical sample of the condensate and found its benzopyrene content to be approximately 0.6 µg. per gramme, a figure within the usual range.

100 male and 100 female stock albino mice of an outbred strain known to be moderately sensitive to skin tumour induction by chemical agents were used for the experiment. Mice of each sex were allotted at random to 5 experimental groups so that each group consisted of 20 males and 20 females. The dorsal hair was removed from mice by electric clippers at the beginning of the experiment and thereafter at 2-3 week intervals. *Group 1* was given thrice weekly applications of 40 mg. smoke condensate in 0.2 ml. acetone (a 20% solution/suspension) to the dorsal skin for 68 weeks. *Group 2* was treated similarly except that the benzopyrene content of the condensate was increased approximately three-fold by the addition of benzopyrene. *Groups 3 and 4* were treated with condensate enriched 10-fold and 50-fold with benzopyrene respectively. In terms of actual amounts of benzopyrene, *Groups 1-4* were given, respectively, 0.025 µg., 0.06 µg., 0.25 µg. and 1.25 µg. benzopyrene at each thrice-weekly application. *Group 5* was treated with the same concentration of benzopyrene as group 4, but the vehicle was acetone only instead of a 20% solution/suspension of smoke condensate in acetone.

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TABLE I

The effect of added 3,4-benzopyrene (BP) on the carcinogenicity of cigarette smoke condensate on mouse-skin

Group	Treatment	Dosage	Incidence of skin tumours at 68 weeks			Incidence of skin tumours at 84 weeks	
			Survivors	Benign tumours	Malignant* tumours	Survivors	Malignant tumours
1	Condensate with « normal » BP content**	40 mg. (as 0.2 ml. of 20 % solution/suspension in acetone) X3 weekly for 68 weeks	31	0	0	26	3
2	Condensate with X3 BP		21	4	0	15	5
3	Condensate with X10 BP		19	0	1	15	2
4	Condensate with X50 BP		24	17	5	14	10
5	BP in acetone (same concentration as in Group 4)	0.2 ml. × 3 weekly for 68 weeks	21	0	0	14	0

* Cumulative total.

** i.e. 2.4 µg BP in condensate from 100 cigarettes (i.e. 4g. condensate)

Table I indicates the results of this experiment. The incidence of benign and of malignant tumours is shown at 68 weeks when treatment was stopped, and also at 84 weeks. Tumours were only regarded as malignant when they had penetrated the panniculus carnosus muscle. The malignant tumours were either squamous or anaplastic carcinomata.

The first point of interest about the results is that smoke condensate alone (Group 1) was more carcinogenic than an acetone solution containing 50 times as much benzopyrene (Group 5). This result indicates quite clearly that the benzopyrene in cigarette smoke condensate cannot, on its own, play more than a *minor carcinogenic role*. One interpretation of the results in Groups 4 and 5 is that benzopyrene in smoke condensate is far more carcinogenic than the same concentration of benzopyrene in acetone. This is precisely as we would have expected from our knowledge of the co-carcinogenic activity of the phenolic fraction of the condensate. It is also confirmatory of GELLHORN's work (5). As stated already, the tumour promoting potentiality of smoke condensate exceeds its initiating potentiality. The fact that increasing the benzopyrene content 3-fold or 10-fold did not lead to a very dramatic increase in carcinogenicity suggests that its importance as a tumour initiator is limited.

The results as a whole indicate that the benzopyrene in smoke condensate plays a negligible role as a complete carcinogen and only a minor role as an initiator of carcinogenesis. It is hoped that this report will direct the attention of chemical investigators to carcinogenic constituents of smoke condensate other than benzopyrene.

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SUMMARY

(1) When 1.25 µg. 3,4-benzopyrene in 0.2 ml. acetone was applied thrice weekly to the dorsal skin of mice for 68 weeks, a few transitory benign

tumours but no malignant tumours appeared. However when mice were similarly treated with a 20 per cent acetone solution of cigarette smoke condensate which contained only 0.025 μ g. 3,4-benzopyrene per 0.2 ml. solution, malignant tumours as well as benign were seen. This result indicated that the carcinogenicity of cigarette smoke condensate for mouse skin could not have been due solely to its 3,4-benzopyrene content.

(2) When 3,4-benzopyrene was added to cigarette smoke condensate so as to increase its concentration to 1.25 μ g. in 0.2 ml., a mixture of high carcinogenic potential was produced. These findings are consistent with the suggestion made earlier that there are co-carcinogenic components in smoke condensate which can magnify the carcinogenic effect of 3,4-benzopyrene.

RÉSUMÉ

1. En appliquant 3 fois par semaine pendant 68 semaines une solution de 1,25 μ g de 3,4-benz-

pyrène dans 0,2 ml d'acétone sur la peau du dos des souris, quelques tumeurs bénignes passagères sont apparues. Aucune tumeur maligne n'a été notée. Si, toutefois, des souris sont traitées de la même façon avec une solution d'acétone à 20% de condensat de fumée de cigarettes, ne contenant que 0,025 μ g de 3,4-benzopyrène par 0,2 ml, des tumeurs malignes apparaissent ainsi que des tumeurs bénignes. Ce résultat indique que la carcinogénicité du condensat de fumée de cigarette appliqué sur la peau de souris ne peut être attribué entièrement à son contenu en 3,4-benzopyrène.

2. Lorsqu'on ajoute du 3,4-benzopyrène au condensat de fumée de cigarette de façon à augmenter sa concentration jusqu'à 1,25 μ g par 0,2 ml, on obtient un produit d'un pouvoir cancérigène élevé. Ces constatations sont en accord avec la suggestion faite précédemment, c.-à.-d. que le condensat de fumée contient des substances cancérigènes, capables d'augmenter l'effet cancérigène du 3,4-benzopyrène.