A Simple Test for Exposure to Polycyclic Hydrocarbons

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The development of a paper chromatographic solvent system capable of selectively separating the carcinogenic hydrocarbon fraction of a high temperature tar mixture has made it possible to undertake the semi-quantitative determination of polycyclic hydrocarbons in human urine from people exposed to these agents (1). The microtechnique described in this paper has been used to evaluate the exposure by inhalation of selected individuals to polycylic hydrocarbons present in the environment. The procedure involves the isolation of a polycyclic hydrocarbon mixture from a urine sample, separation of the hydrocarbons by paper chromatography, and semi-quantitative determination of the amount of certain hydrocarbons by measuring the horizontal diameter of the ultraviolet fluorescent spots, in particular that due to 1,2,3,4- dibenzopyrene.

Extraction Technique

Urine samples were usually 600ml for smokers and 1200 ml for non-smokers or passive smokers. By passive smokers is meant one who does not voluntarily smoke but who is compelled by certain circumstances to both inhale and reinhale the cigarette smoke generated by on or more smokers.

To 600ml of urine, 120 ml of concentrated hydrochloric acid was added and the mixture refluxed for 90 minutes on a water bath. During the last 30 minutes, 6 grams of granular metallic zinc was gradually added through the reflux condenser. When the flask was completely cooled, the condenser was rinsed with petroleum ether and the mixture transferred to an onion shaped flask, Fig. 1.

The acid urine solution was extracted with three portions of petroleum ether and the separated combined ether layers were shaken with two normal sodium hydroxide. The sodium hydroxide layer was removed, extracted twice with petroleum ether and the petroleum ether solutions added to the original petroleum ether extract (about 1000 ml). The extract was carefully evaporated to dryness and the remaining solids extracted 10 times with small portions of cold methanol, each extraction taking 15 minutes. The combined methanol solutions were filtered and transferred to a special evaporation vessel, Fig 2.

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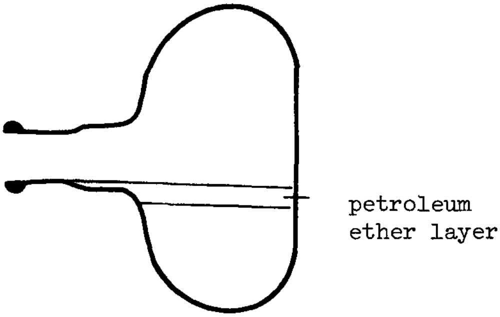

Fig 1.

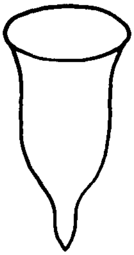

Fig 2.

Upon complete evaporation of the methanol the inside walls of the vessel were rinsed with small amounts of petroleum ether and benzene. The solvent, now contained in the tip of the vessel, was evaporated and the remaining solids dissolved in 0.05 to 0.1 ml of benzene.

Part of the residue was immiscible in benzene and formed a small layer under the solvent. Aliquots of the benzene solution were used for paper chromatographic separation.

Paper Chromatographic Technique

The paper chromatographic procedure has been described previously (2,3). Ascending development of the chromatogram was complete in six hours at room temperature. Separations were less complete when Whatman # 4 paper was employed.

Ultraviolet examination of the chromatogram revealed a spot for 1,2,3,4-dibenzopyrene whose Rf value was comparable to that obtained with an authentic specimen and to that obtained from a chromatogram of a high temperature tar mixture. Other spots which could be identified included those due to 3,4-benzfluoranthene, fluoranthene, and 3,4-benzopyrene, Fig 3.

The quantity of each polycyclic hydrocarbon was estimated by comparing the diameter of the spot with a calibration curve similar to that of 1,2,3,4-dibenzopyrene shown in Fig 4.

Results and Discussion

To illustrate the applicability of this procedure for the determination of polycyclic hiydrocarbons in the urine of smokers or workers exposed to airborne particulates containing high temperature tar, the following example is presented.

Urine from an active smoker and a passive smoker, both sitting 8 hours per day in a closed room of 35 cubic meters volume, were examined by the procedure described above. The results of this examination are presented in Tables 1 & 2.

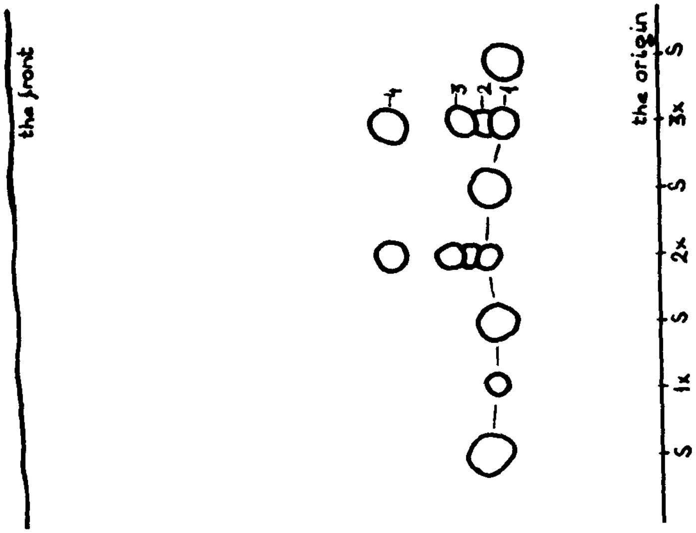

Fig 3. 1,2,3,4-dibenzopyrene standard applied at points S. Benzene extract applied at other points: 1X = 1.3µl; 2X = 2.6µl ; 3X = 3.9µl. Spot 1 is 1,2,3,4-dibenzopyrene; spot 2 is 3,4-benzopyrene; spot 3 is 3,4-benzofluoranthene; spot 4 is fluoranthene.

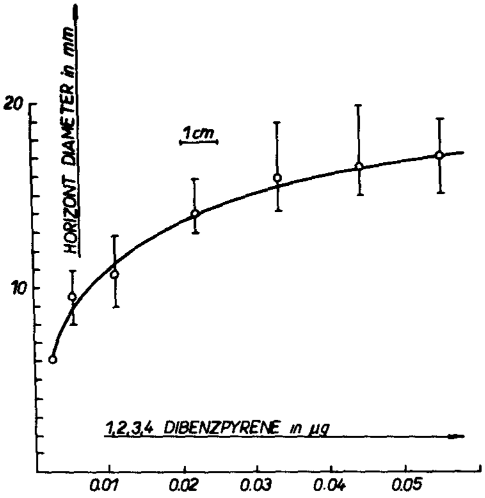

Fig 4.

Table 1

aliquot applied in µl	horizontal diameter of spot in mm	1,2,3,4-dibenzopyrene µg
1.3	9.5	0.0065
2.6	10.5	0.0120
3.9	14.0	0.0220

Table 2

		1,2,3,4-dibenzopyrene µg/L
Passive smoker	Simultaneous urine sample with that of active smoker	0.3
	Two weeks after removal from exposure	0.0
Active Smoker		1.1

The level of 1,2,3,4-dibenzopyrene in the urine of the passive smoker was considerably less than that in the active smoker but nevertheless was a significant amount. Removal of the passive smoker from this environment caused a marked decrease in the amount of 1,2,3,4-dibenzopyrene in the urine.

It appears that the procedure described is useful for sceening individuals exposed to polycyclic hydrocarbons. The fluoresence of 1,2,3,4-dibenzopyrene is strong and stable and it is metabolized to a lesser extent than 3,4-benzopyrene (4). The extraction procedure reduces to a minimum background interferences coming from non-polycyclic hydrocarbon components of the urine and consequently results in greater analytical sensitivity.

References

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2. MALÝ, E. *J. Chromatog.* **7**, 422 (1962), *Ibid.* **40**, 190 (1969).

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