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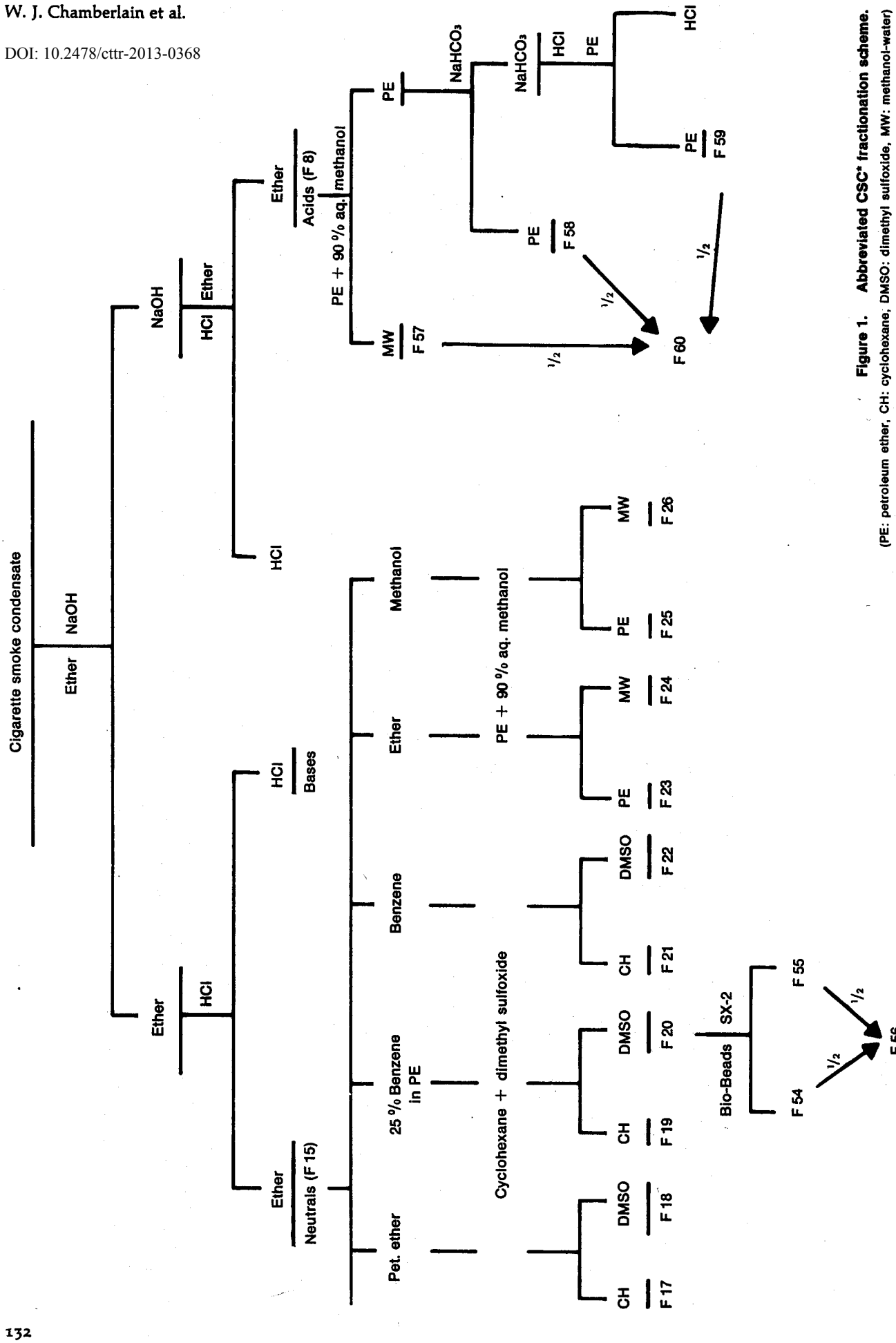

Figure 1. Abbreviated CSC* fractionation scheme.

(PE: petroleum ether, CH: cyclohexane, DMSO: dimethyl sulfoxide, MW: methanol-water)

* Cigarette smoke condensate.

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Fractionation of Cigarette Smoke Condensate for Biological Testing

Concentration of Polynuclear Aromatic Hydrocarbon and Weak-Acid Fractions

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INTRODUCTION

Our studies to relate by mouseback testing the tumorigenicity of cigarette smoke condensate (CSC) to that of its active components are continuing. The large-scale fractionation of CSC (1), the subfractionation of the CSC neutrals by chromatography on silicic acid (2), and various solvent partition studies on the neutrals (3) have been reported. Results of the bioassays on the fractions prepared in these studies have also been reported (4, 5, 6). The tumorigenic activities of three CSC fractions have been shown to account for most of the CSC activity. These fractions are the ether-soluble weak-acid fraction (F8), a polynuclear aromatic hydrocarbon-containing fraction (F20), and a polar neutral fraction (F26) (Fig. 1). Recombinations of these active fractions have reproduced the initial activity of crude CSC (6). In the present study, the methods and yields obtained in further fractionating fractions F8 and F20 are reported.

Currently, the polynuclear aromatic hydrocarbons (PAH) are being subjected to analysis and a report on PAH identification will be presented in this journal in the near future.

MATERIALS AND METHODS

All solvents used were Burdick and Jackson** distilled-in-glass grade. The CSC was prepared in 1.0 kg batches at the Roswell Park Memorial Institute and shipped to Athens under conditions previously described (1). A total of 10 kg of CSC was fractionated in this experiment. Fractions F8 and F20 were prepared according to a previously described procedure (2).

Before subfractionation of F8, a 25% portion of the total F8 solution was removed as a bioassay control. After removing the solvent, the remaining F8 was partitioned between equal volumes of petroleum ether (PE) and 90% methanol-water (MW). The MW-soluble portion was designated F57, and represented about 74% of F8 or about 6% of CSC. The PE layer was next extracted with aqueous sodium bicarbonate to yield a PE fraction (F58) and an aqueous layer. Fraction F58 contained on the average 1.5% of CSC. The aqueous layer was adjusted to pH 1.0 with 6N HCl and extracted with petroleum ether to yield F 59, representing about 0.5% of CSC. Fifty percent of each of the solutions of these three fractions was removed and combined to form F 60 (reconstituted F 8).

Fraction F20, after removal of 25% for control, was separated by gel permeation chromatography (GPC) on Bio-Beads SX-2 (Bio-Rad Laboratories, molecular exclusion range 2700), with acetone as the eluting solvent. Generally, 0.5 g of F20 was separated on 70 g of Bio-Beads. Two fractions, F54 and F55, were eluted (Fig. 2). The first 150 ml of eluate were designated F54 and contained 85% of the weight of F20, or about 0.3% of CSC. The presence and identity of the PAH in F 55 were established by a combination of GPC and subsequent gas chromatography-mass spectrometry of the highly refined PAH eluate (7, 8). Again, 50% each of F54 and F55 were recombined to yield F56 (reconstituted F20). Yields of individual fractions (Tables 1 and 2) represent values obtained from ten individual 1 kg quantities of CSC fractionated over a one-year period.

In the course of the experiment, the other biologically active fraction of CSC, F26, was also obtained and 25% of F26 was removed and submitted for bioassay as a check on our previous bioassay studies. The remainder was used for identification studies and yields for F26 are included in Table 2.

* Received for publication: 28th November, 1974.

** Mention of commercial items does not imply their endorsement by the Department over similar products not mentioned.

Table 1. Average yields[a] of weak-acid fractions.

Fraction	Range (g)		Mean (g)	Standard deviation
	Min.	Max.		
F 8[b]	39.48	81.28	61.56	12.32
F 8[c]	16.03	26.42	20.67	3.18
F 57	15.39	29.68	22.23	4.33
F 58	3.42	8.93	5.71	2.14
F 59	1.27	2.81	1.86	0.51
F 60	19.00	53.04	32.65	9.50

[a]Based on 1000 g of CSC.
[b]Used for fractionation, represents 75 % of total F 8.
[c]Used as control, represents 25 % of total F 8.

Table 2. Average yields[a] of neutral subfractions.

Fraction	Range (g)		Mean (g)	Standard deviation
	Min.	Max.		
F 15	220.17	260.39	240.85	15.51
F 20[b]	2.02	3.19	2.62	0.45
F 20[c]	0.62	1.17	0.91	0.19
F 54	0.77	1.24	1.01	0.16
F 55	0.11	0.21	0.15	0.05
F 56	0.81	1.27	1.07	0.16
F 26	15.64	19.88	17.44	0.72

[a]Based on 1000 g of CSC.
[b]Used for fractionation, represents 75 % of total F 20.
[c]Used as control, represents 25 % of total F 20.

RESULTS AND DISCUSSION

As stated previously (6), our objectives have always been to prepare fractions in such a way that all fractions can be tested and tested in the relative proportions in which they were obtained from CSC. This procedure should lead to a number of fractions which represent a small percentage of CSC, while still containing all its activity. So far, we have shown that the bulk of CSC was inactive as a tumor promoter on mouse skin, with activity concentrated in about 10% of the weight of CSC (4, 5, 6). The active fractions F20, F26, and F8 represented 0.35%, 2.25%, and 8.0% of CSC, respectively. The abbreviated CSC fractionation scheme, without experimental details, is presented in Figure 1.

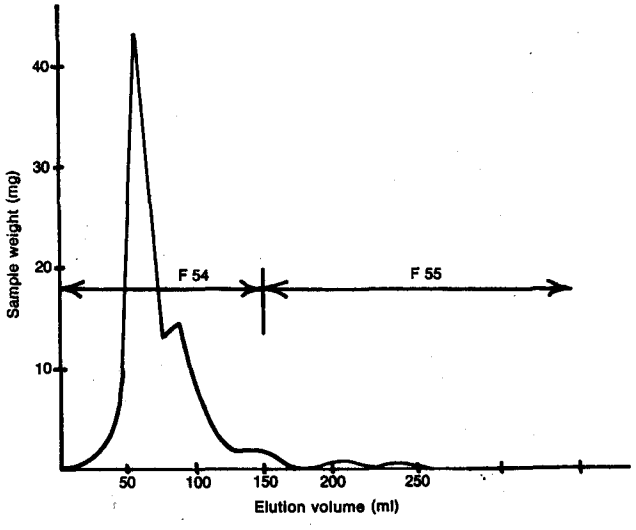
The objective of this study was to further fractionate F20 (PAH) and F8 (weak acids) in order to concentrate the activity in a still smaller percentage of the initial weight of CSC. Such concentration would also facilitate identification of the active components. Using GPC techniques, the PAH have been successfully concentrated into F 55, which represents about 15% of F 20 or only 0.05% of CSC. The weight distribution curve for the gel chromatography of F20 on SX-2 Bio-Beads (Fig. 2) shows that non-PAH material, removed in F54, accounted for at least 85% of F20 and the PAH have been concentrated six-fold.

The purpose of fractionating F8 was to concentrate the free fatty acids as one group of compounds. Previously, the non-volatile weak acids, possibly fatty acids, had shown activity as promoting agents (9). Thus, F8 was first partitioned between PE and 90% methanol-water to isolate the PE-soluble fatty acids, while removing the more polar acids into F57. The PE-soluble acids, representing about 25% of F8, were subsequently treated with aqueous sodium bicarbonate in order to extract the free fatty acids. Acidification of the basic solution yielded a fatty acid-enriched fraction F59, which represented only 0.5% of CSC. In this manner, the fatty acids were concentrated 16-fold. Other PE-soluble acidic material remained in F58 (1.5% of CSC). Tables 1 and 2 give average weights of each fraction from the fractionations of ten individual kilograms of CSC. As previously observed (6), the variability in yields of some of the fractions was quite high. Consequently, the mean weight does not represent the exact percentage of the fraction in CSC. Numerous factors, including the preparation of different batches of CSC over a one-year period and different scientists performing the fractionation, may account for the observed variability. The bioassay tests are nearing completion and the results will be reported shortly. At that time, the nature or identity of any tumor-promoting material in these fractions will be discussed fully.

SUMMARY

Cigarette smoke condensate (CSC) was fractionated for bioassay to determine possible tumorigenic activity on mouse skin. Two fractions which previously had shown activity were further separated. A weak-acid fraction (F8) was separated into three subfractions. A polynuclear aromatic hydrocarbon-containing fraction (F20) was divided into two fractions by gel permeation chromatography. The polynuclear aromatic hydrocarbons, the suspected active materials in F20, were successfully concentrated into a fraction (F55) representing only 0.05% of CSC. These materials are currently undergoing bioassay.

Figure 2. Separation of F 20 on Bio-Beads SX-2 in acetone.



ZUSAMMENFASSUNG

Cigarettenrauchkondensat (CSC) wurde für biologische Untersuchungen fraktioniert, um die mögliche cancerogene Aktivität auf der Mäusehaut zu bestimmen. Zwei Fraktionen, die vorher eine Aktivität gezeigt hatten, wurden weiter getrennt. Eine schwach saure Fraktion (F 8) wurde in drei Unterfraktionen aufgetrennt. Eine Fraktion (F 20), die polycyclische aromatische Kohlenwasserstoffe enthielt, wurde durch Gel-Chromatographie in zwei Fraktionen aufgeteilt. Die polycyclischen aromatischen Kohlenwasserstoffe, auf denen vermutlich die Aktivität der Fraktion F 20 beruht, wurden erfolgreich in einer Fraktion (F 55) konzentriert, die nur 0,05 % des Cigarettenrauchkondensates ausmachte. Diese Fraktion wird gegenwärtig im biologischen Test untersucht.

RESUME

Le condensat de fumée de cigarette a été fractionné en vue d'essais biologiques pour déterminer l'action tumorigène possible sur de la peau de souris. Deux fractions, qui avaient montré précédemment une certaine activité, ont été refractionnées. Une fraction faiblement acide (F 8) a été séparée en trois sous-fractions. Une fraction F 20, contenant des hydrocarbures polynucléaires aromatiques, a été séparée en deux fractions par chromatographie à perméation de gel. Les composés suspectés d'activité de la F 20, des hydrocarbures polynucléaires aromatiques, ont pu être concentrés en une fraction (F 55) ne représentant que 0,05 % du condensat de fumée. Les tests biologiques sont en cours.

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