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CONFIDENTIAL WORK PRODUCT

RESEARCH AT OHIO STATE UNIVERSITY

This report outlines some of the experimental details of the research conducted at Ohio State University. Historical aspects of the research can be found in a November 27, 1985 memo. In brief, Lorillard provided funds to the Ohio State Research Foundation, which in turn funded research by Mr. John Fishel, a graduate student at the Ohio State University. Dr. Joseph Haskins was the supervising professor of the project. The lab work took place between June 16, 1946 and March 30, 1948. Chemical analysis of cigarette smoke was attempted.

Dr. Haskins' approach and comments provide insight into the state of the art in the 1940s and before. His and Mr. Fishel's analysis was that although

[T]he literature contains a number of analyses of tobacco smoke, but some objection may be raised to the procedures followed in each case. For example, Neuberg's work, which is the most extensive, was done by smoking tobacco in large quantities in a pipe . . . with a constant vacuum rather than a constant volume.¹

Haskins also critically evaluated analytical methods.² Many of the results reported in earlier work were based on nonspecific methods. A nonspecific method is, for example, one based on the reducing properties of a compound rather than the specific elemental substance. It was noted by Dr. Haskins that in the OSU studies, smoke was collected from cigarettes smoked in a manner

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simulating human smoking. Dr. Haskins tried to determine the amounts of specific components, whereas some earlier work was more qualitative.

Dr. Haskins also commented on a public article regarding smoking and lung cancer. He was skeptical that there was a higher percentage of cancer among smokers.³ He specifically commented on Dr. Grace's work published in Medical Times⁴ and the American Journal of Surgery.⁵ He mentioned that he had information on Roffo's identification of benzpyrene as a constituent of tar.^{6,7} Both Roffo and Grace were cited by plaintiff's expert, Jeffrey Harris.

A. The Smoking Machine.

The smoking machine was constructed at OSU. The design was from the work of Bradford, Harlan, and Hamner (1936).⁸ Several modifications were made to allow continuous or long-term operation. The machine could smoke four cigarettes at once. Each was puffed for two seconds, once a minute, with a 40 cc. puff volume. Two hundred cigarettes could be smoked in an eight-hour day.⁹

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B. Separation of the Particulate and Gas Phase.

Investigations prior to 1946 had separated the two phases by a number of methods. Mr. Fishel tried several methods, but never settled on one preferred method. In one method, the smoke was passed through a glass tube 3 cm. in diameter and 30 cm. long, packed to a depth of 23 cm. with glass helices 1/8 of an inch in diameter each having a single turn. Smoke particles were adsorbed on the surface of the glass helices. Most of the analytical work was performed on tar or gases separated in this manner.¹⁰

1. Examination of the Gas Phase.

It was planned to make determinations of carbon monoxide, carbon dioxide, hydrogen sulfide, and hydrogen cyanide. Several analytical methods were used for each compound. All the methods employed wet analytical chemistry or gravimetric techniques. The determinations for the latter gases were fairly straightforward; however, problems arose in the determination of carbon monoxide. In general, the previously separated gas was bubbled through reagent solutions or passed through an absorption train. The absorption train for determination of carbon monoxide was as follows:

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- (1) A bubbler tube containing a saturated solution of silver sulfate in 1:1 sulfuric acid (to remove HCl and H₂S).
- (2) Drierite in a midvale bulb to remove moisture.
- (3) Ascarite in a midvale bulb to remove carbon dioxide.
- (4) A combustion tube 12 inches long and 3/4 inches in diameter containing a 4-1/2 inch plug of copper oxide wire, heated by a Bunsen burner with a wing top, to a red heat.
- (5) Drierite in a midvale bulb to collect any water formed.
- (6) Ascarite in a midvale bulb to absorb the carbon dioxide formed.
- (7) Mixed-ascarite drierite in a midvale bulb to prevent absorption of moisture or carbon dioxide from the air.¹¹

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Cigarette smoke was passed through the separating apparatus and then along the absorption train. In Steps 1 through 3, everything but carbon monoxide should have been removed. At Step 4, the carbon monoxide would be oxidized to carbon dioxide. If any gaseous hydrocarbons were present, they would also be oxidized to carbon dioxide and water. Significant quantities of water and carbon dioxide were found in the respective bulbs in Steps 5 and 6. This indicated that some gaseous compound containing hydrogen was coming over with the carbon monoxide. Not all of the carbon dioxide found in Bulb 6 could be attributed to carbon monoxide. The presence of gaseous hydrocarbons was suspected. Another trap, immersed in liquid air, was inserted in the absorption train between Steps 3 and 4 above.¹² This cold trap removed an unidentified white solid and resulted in lower weights of carbon dioxide and water. The continued presence of water indicated there were still impurities. Dr. Haskins was never fully satisfied that the amount of carbon monoxide was accurately determined.

Subsequently, the white solid captured in the cold trap was analyzed. Upon gradual warming, the white solid vaporized and was passed through the remaining absorption train. The results of this analysis indicated that acetylene was present. It was also noted that fractions of this material had the characteristic odor of unsaturated hydrocarbons, such as cyclohexidene

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or butadiene. Attempts to identify butadiene were unsuccessful.

Dr. Haskins considered the identification of acetylene as a major accomplishment. According to his July 1948 report, this was the first evidence of the presence of such compounds, i.e., acetylene and other unsaturated gaseous hydrocarbons.

Mr. Fishel and Dr. Haskins reported the amount of carbon monoxide to be between 2.1 and 3.9 ml. per cigarette, or 0.48% to 0.9% by volume. Values reported in the literature from 1900 to 1940 ranged from 2.6 ml. to 30 ml. per cigarette. Dr. Haskins felt these results were a major accomplishment. He thought it likely that a more detailed investigation might show the amount of carbon monoxide to be much less.

Carbon dioxide was determined by a method similar to the method used for carbon monoxide. The gas was passed through an absorption train. A bulb containing ascarite absorbed the carbon dioxide from the gas. The bulb was then weighed and the increase in weight was the weight of carbon dioxide in the gas. Hydrogen sulfide, acetylene, and hydrogen cyanide were determined by bubbling the gas through an appropriate reagent solution. Classical wet analytical techniques were then employed to determine the amounts of the respective compounds. The method chosen for hydrogen cyanide was believed to be the most specific and

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sensitive method available.¹³ No hydrogen cyanide was detected. The other analyses were usually performed on the smoke from five to twenty-five cigarettes. The number of cigarettes used in the hydrogen cyanide determination was not reported. Previous investigators had reported that cyanide was present. Dr. Haskins suspected that any hydrogen cyanide present may have been removed by the tar trap.

2. Final Results of the Gas Phase Analysis.¹⁴

<u>Specific Components</u>	<u>Analytical Results</u>
Carbon Dioxide	7.77% by volume
Carbon Monoxide	0.48-0.9% by volume
Hydrogen Sulfide	0.0019% by volume
Acetylene	5 mg. per 100 cigarettes
Hydrogen Cyanide	None detected

C. Analysis of the Particulate Phase.

Forty grams of tar were collected by January 1947. It was planned to separate the tar into broad classes of components, then attempt to determine the amounts of individual components within each class. Specific results were not obtained. Dr. Haskins attributed this failure to a lack of a satisfactory process of separation and the mistake of not starting with a larger amount of tar.¹⁵

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The separation scheme was a steam distillation technique outlined by Neuberg in 1931.¹⁶ It separated the tar into basic components (nicotine and other alkaloids), carboxylic acids, aldehydes, and ketones. Attempts were made to prepare derivatives of the ketones and carboxylic acids. Individual components could not be isolated by fractional recrystallization or by column chromatographic techniques. A mixture of the phenylphenacyl derivatives of acetic, propionic, and butyric acids were prepared. This mixture was separated on a column of silicic acid and gave distinct bands which fluoresced under ultraviolet light. Mr. Fishel was unable to separate the carboxylic acid fraction in an analogous manner.

In suggesting future work, Dr. Haskins proposed a different approach. This was to collect tar in a toluene solution, then make neutral, acidic, and alkaline aqueous extractions in a separatory funnel. The subsequent manipulations would be similar to what was previously attempted, i.e., make aromatic derivatives so that ultraviolet fluorescent bands could be isolated on chromatographic columns. Again, the attempt would have been to identify the same broad classes as above. No mention was made of any future attempts to identify other components, such as PAH.

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D. Developments in the State of the Art.

Progress in methodology was rather slow from 1900 to 1955. A few significant contributions were made in the 1950s, using techniques similar to classical methods. After 1958, gas chromatographic methods revolutionized tobacco smoke analysis.¹⁷ Fishel and Haskins identified the first volatile hydrocarbon in 1947. To illustrate the advance in progress, consider the following: Wynder and Hoffman in their book, Tobacco and Tobacco Smoke (1967), reported that 14 volatile hydrocarbons had been identified by 1959, and that by 1965 over 55 had been identified.¹⁸ Mr. Fishel compiled a reference list of smoke constituents that had been identified with reasonable certainty up to 1945.¹⁹ There were only 70 entries on the list. One of the entries on the list was benzpyrene. Wynder did not believe that accurate determination of benzpyrene occurred much before 1960.²⁰ Reliable and reproducible determinations of most of the components studied at Ohio State University did not appear before 1960. However, the OSU figures are a fairly good ballpark estimate. The following table roughly compares results obtained at OSU to results reported by Wynder and Hoffman in 1967. Exact comparisons cannot be made because of the variations in cigarette design, and methods of smoke formation and collection.

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<u>Components</u>	<u>OSU</u>	<u>Wynder & Hoffman</u>
Carbon Dioxide	7.77%	8-10 volume %
Carbon Monoxide	0.48-0.96%	2.7-6.0 volume %
Acetylene	5 mg./100 Cig.	3.0 mg./100 Cig.
Hydrogen Sulfide	1.18 mg./100 Cig.	2.7 mg./100 Cig.
Hydrogen Cyanide	None detected	3.2-11.5 mg./100 Cig.

(Cig. = cigarettes)

E. Summary.

The research conducted at Ohio State University from 1946 to 1948 was primarily a chemical analysis of cigarette smoke. Chemical analysis of complex mixtures during this time period was a difficult, time-consuming task. Dr. Parmele of Lorillard seemed satisfied with the work of Mr. Fishel. He was dissatisfied with the supervision supplied by Dr. Haskins. Mr. Fishel decided to leave Ohio State University on April 1, 1948.²¹ Dr. Parmele did not recommend the continuation of the project with another graduate student under the supervision of Dr. Haskins. Lorillard's association with Ohio State University thus ended.

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