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The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites

I. Introduction*

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The quantitative analysis of carcinogenic hydrocarbons and derivatives in tissue extracts by ultraviolet spectrophotometry has not been developed to any considerable extent, although both Chalmers (5) and Lorenz and Shear (21) demonstrated several years ago that such analysis is practicable. Polynuclear aromatic hydrocarbons, including alkyl and alicyclic derivatives, possess intense and very characteristic spectra which have been used frequently to establish the structure of synthetic compounds (11-14). The observation of Shear (25) that a tumor can be initiated in a mouse by as little as 0.4 μgm. of 1,2,5,6-dibenzanthracene dissolved in cholesterol tended to discourage further investigations along these lines for some time, as such quantities are close to the limit of sensitivity of spectrographic analysis even under optimum conditions, and it seemed improbable that spectrometric studies could contribute much to an understanding of the mechanism of tumor initiation by carcinogens if such minute quantities of hydrocarbon are instrumental in initiating the proliferation of cancerous cells.

Recently considerable interest has been shown in the detoxification processes by which hydrocarbon carcinogens are excreted (1, 2, 4, 6, 8) and which may play a role in the removal of the hydrocarbon from the site of injection. In this type of investigation quantities of the order of milligrams of hydrocarbon are involved, and, provided certain technical difficulties can be overcome, spectrophotometric analyses of hydrocarbons in tissue extracts and excreta extracts might yield valuable information concerning the mechanism of detoxification and enable the influence of dietary and other factors on the rate and mode of excretion to be studied quantitatively.

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TECHNIC OF SPECTROPHOTOMETRIC ANALYSIS

Most methods of quantitative spectrographic analysis involve the use of a rotating sector or some equivalent optical device by which the ratio of the intensity of monochromatic light I transmitted by a given length of the solution is compared with that transmitted by a cell of the same length containing the solvent only, I_0. This comparison is made over a range of wave lengths. The apparatus used in this laboratory has been described previously (16).

The molecular extinction coefficient ϵ is defined by the equation

$$\epsilon = \frac{1}{c \cdot l} \cdot \log \frac{I_0}{I}$$

where l is the cell length in cm., c the concentration of the absorbing solute in moles per litre, and $\log I_0/I$, the density. Where the molecular weight of the solute is not known or where the solute is not a pure substance, ϵ cannot be evaluated and in such cases it is convenient to use the function $E^{1\%}_{1\text{ cm.}}$ which is defined as

$$E^{1\%}_{1\text{ cm.}} = \frac{1}{w \cdot l} \cdot \log \frac{I_0}{I}$$

where w is the concentration of the solute in gm. per 100 ml. of solution.[1] It will be evident that in the case of a pure absorbing solute

$$E^{1\%}_{1\text{ cm.}} = \frac{10\epsilon}{m}$$

where m is the molecular weight of the solute.

If the solute is a tissue extract containing non-absorbing material as well as absorbing constituents, the observed value of $E^{1\%}_{1\text{ cm.}}$ at a given wave length

[1] The symbols and nomenclature used here have recently been standardized by a joint committee of the *Journal of Biological Chemistry* and the Optical Society of America.

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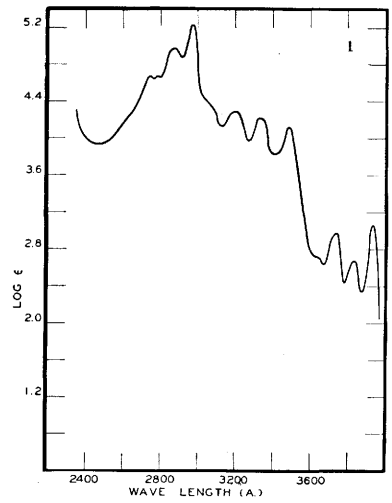

FIG. 1.—1,2,5,6-Dibenzanthracene (solvent ethanol).

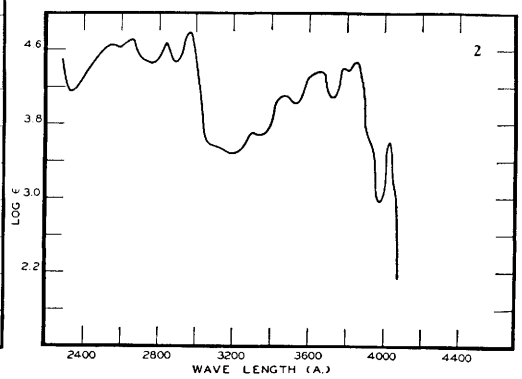

FIG. 2.—3,4-Benzpyrene (solvent ethanol).

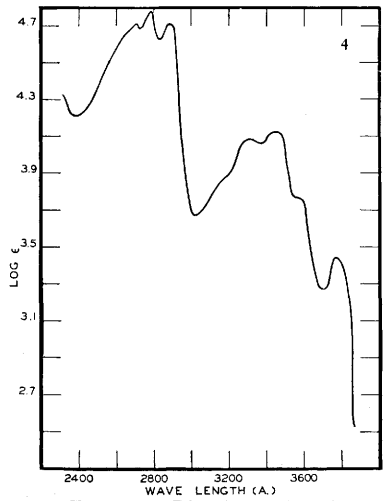

FIG. 4.—4,9-Dimethyl-5,6-benzthiophanthrene (solvent ethanol).

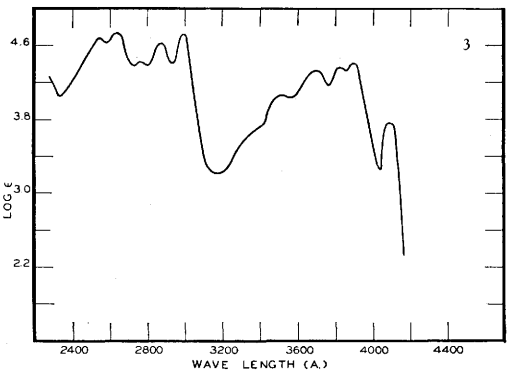

FIG. 3.—2-Methyl-3,4-benzpyrene (solvent ethanol).

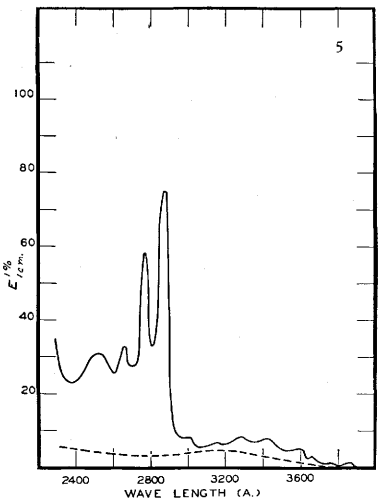

FIG. 5.—Mouse liver extract, nonsaponifiable fraction. *A*, continuous line ————, with 1,2-benzanthracene added. *B*, dash line – – – –, control.

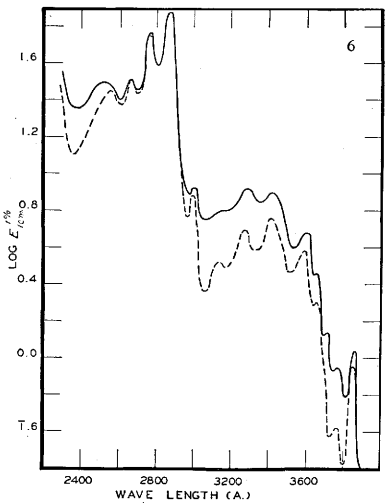

FIG. 6.—*A*, continuous line ————, mouse liver extract, nonsaponifiable fraction with added 1,2-benzanthracene. *B*, dash line – – – –, master curve of 1,2-benzanthracene.

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will be less than that for the pure absorbing substance and the percentage concentration (c_1) of the absorbing constituent in the solute will be given by the equation

$$c_1 = \frac{E^{1\%}_{1\text{ cm.}\ \text{observed}}}{E^{1\%}_{1\text{ cm.}\ \text{pure solute}}} \times 100\ \%$$

1,2,5,6-dibenzanthracene, 3,4-benzpyrene, 3,4-benzphenanthrene, chrysene, and their respective alkyl and alicyclic derivatives.

The absorption spectrum of 1,2-benzanthracene in ethanol was measured first by Capper and Marsh (3) and later by Mayneord and Roe (22) who have also

TABLE I: REFERENCES TO ABSORPTION SPECTRA OF CARCINOGENIC HYDROCARBONS AND RELATED COMPOUNDS

Compound	Solvent	References
1,2-Benzanthracene	Ethanol	3, 16, 22
1′-Methyl-1,2-benzanthracene	Ethanol	16
4-Methyl-1,2-benzanthracene	Ethanol	16
5-Methyl-1,2-benzanthracene	Ethanol	16
6-Methyl-1,2-benzanthracene	Ethanol	22
7-Methyl-1,2-benzanthracene	Ethanol	22
8-Methyl-1,2-benzanthracene	Ethanol	16
9-Methyl-1,2-benzanthracene	Ethanol	16
10-Methyl-1,2-benzanthracene	Ethanol	16
6-Isopropyl-1,2-benzanthracene	Ethanol	23
10-Isopropyl-1,2-benzanthracene	Ethanol	22
5,8-Dimethyl-1,2-benzanthracene	Ethanol	16
5,10-Dimethyl-1,2-benzanthracene	Ethanol	16
6,7-Dimethyl-1,2-benzanthracene	Ethanol	22
8,10-Dimethyl-1,2-benzanthracene	Ethanol	16
9,10-Dimethyl-1,2-benzanthracene	Ethanol	16
3′-Isopropyl-10-methyl-1,2-benzanthracene	Ethanol	17
5,6-Cyclopenteno-1,2-benzanthracene	Ethanol	22
6,7-Cyclopenteno-1,2-benzanthracene	Ethanol	22
Cholanthrene	Ethanol	12, 23
6-Methylcholanthrene *	Ethanol	17
20-Methylcholanthrene *	Ethanol	22
6,20-Dimethylcholanthrene *	Ethanol	17
6,22-Dimethylcholanthrene *	Ethanol	17
1′,9-Methylene-1,2-benzanthracene	Ethanol	16
10-Methyl-1′,9-methylene-1,2-benzanthracene	Ethanol	17
1,2,5,6-Dibenzanthracene	Benzene	7
1,2,5,6-Dibenzanthracene	Ethanol	5, 22 This paper, Fig. 1
1,2,5,6-Dibenzanthracene	Chloroform	5
1,2,5,6-Dibenzanthracene	Ether	21
2′-Methyl-1,2,5,6-dibenzanthracene †	Ethanol	2
3′-Methyl-1,2,5,6-dibenzanthracene †	Ethanol	2
9,10-Dimethyl-1,2,5,6-dibenzanthracene	Ethanol	22
Chrysene	Ethanol	22
5-Methylchrysene	Ethanol	18
4,5-Dimethylchrysene	Ethanol	18
5,6-Dimethylchrysene	Ethanol	18
4,5-Methylenechrysene	Ethanol	18
3,4-Benzpyrene	Ethanol	22 This paper, Fig. 2
2-Methyl-3,4-benzpyrene	Ethanol	This paper, Fig. 3
8(or 9?)-Methyl-3,4-benzpyrene	Ethanol	24
3,4-Benzphenanthrene	Ethanol	23
4,9-Dimethyl-5,6-benzthiophanthrene	Ethanol	This paper, Fig. 4
4′,8′-Dihydroxy-1,2,5,6-dibenzanthracene	Ethanol	4
Isomeric dihydroxy-1,2,5,6-dibenzanthracene from rabbit urine	Ethanol	2

* Ring numbering according to Fieser's system (see Reference 12).
† Described but no numerical data given.

ABSORPTION SPECTRA OF PURE HYDROCARBONS

Accurate data on the absorption spectra of the pure compounds is a primary requisite for the analysis of carcinogenic hydrocarbons and related substances in tissue extracts. In Table I, references have been summarized relating to the spectra of 1,2-benzanthracene, published accurate spectrographic curves of numerous polynuclear aromatic hydrocarbons (23). Jones (16) has also measured the absorption spectrum of 1,2-benzanthracene in ethanol as well as the spectra of several derivatives (17). Reference should be made to his paper for a comparison of his intensity data for

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1,2-benzanthracene with those obtained by Mayneord and Roe.

Clar (7) recorded the spectrum of 1,2,5,6-dibenzanthracene in benzene solution, an unfortunate choice of solvent as benzene has strong absorption at wave lengths less than 2,700 A. Clar's curve is not plotted in a manner which is readily convertible to molecular extinction coefficients. The curve published by Chalmers (5) illustrates only a small section of the spectrum in the region between 2,700 and 3,100 A., the intensities being given for a 0.0005 per cent solution and not calculated as ϵ or $E^{1\%}_{1\ cm.}$. A complete curve for an ethereal solution is given by Lorenz and Shear (21) and for an alcoholic solution by Mayneord and Roe (22). In neither case is the curve accompanied by a table of the intensity values of the maxima, and significant errors may be introduced in interpolation. The

Table II: Absorption Spectrum of 1,2,5,6-Dibenzanthracene; Wave Lengths and Intensities of the Maxima

Jones * (ethanol)		Mayneord and Roe (ethanol)		Lorenz and Shear (ether)	
λ	log ε	λ	log ε	λ †	log ε
2740	4.68	2750	4.7	....	...
2780	4.68	2780	4.7	....	...
2875	4.98	2875	5.0	2880	5.3
2970	5.24	2970	5.2	2970	5.8
3200	4.29	3200	4.3	3205	4.3
3330	4.23	3330	4.2	3355	4.2
3485	4.13	3490	4.2	3500	4.1
3650	2.70	3630	2.7	....	...
3740	2.98	3730	3.0	3745	3.2
3835	2.68	3830	2.6	3850	2.5
3930	3.06	3930	3.1	3970	3.2

* m.p. = 265-266.5° (corr.).
† Converted from wave numbers.

absorption curve for 1,2,5,6-dibenzanthracene in absolute ethanol, as determined by the present author, is given in Fig. 1, and in Table II the intensities of the maxima are compared with the values interpolated from the curves of Mayneord and Roe (22, 23) and of Lorenz and Shear (21). The curve of the latter authors is more intense than the other two in the region of the highest intensity maxima. This difference may be accounted for in part by the change of solvent, and it should be emphasized that such a change will alter the intensities of the maxima in almost all cases by a significant amount; in solvents of low dielectric constant such as hexane and ether, higher intensities and more resolution of the structure of the bands are observed than in more polar solvents such as ethanol, methanol, or water.

The intensities of the maxima are influenced to some extent by the resolving power of the spectrograph. A slit-width of 0.12 mm. was used in all measurements made by the author.

The spectrum of 3,4-benzpyrene in ethanol has been measured both by Mayneord and Roe and by the present author, and the data are compared in Table III. The spectrum of 2-methyl-3,4-benzpyrene, the high carcinogenic activity of which has recently been demonstrated [2] and of the carcinogenic sulfur-containing substance, 4,9-dimethyl-5,6-benzthiophanthrene are given in Figs. 3 and 4 and Tables IV and V.

Table III: Absorption Spectrum of 3,4-Benzpyrene; Wave Lengths and Intensities of the Maxima

Jones * (ethanol)		Mayneord and Roe † (ethanol)	
λ	log ε	λ	log ε
2545	4.66	2540	4.6
2660	4.70	2650	4.7
....	...	2750	4.45
2840	4.68	2840	4.65
2965	4.78	2960	4.8
3300	3.71	3280	3.7
3465	4.12	3460	4.1
3655	4.38	3640	4.4
3785	4.41	....	...
3850	4.48	3840	4.4
4030	3.62	4030	3.6

* m.p. = 179-179.5° (corr.).
† Referred to by Mayneord and Roe as 1,2-benzpyrene.

Table IV: Absorption Spectrum of 2-Methyl-3,4-Benzpyrene *; Wave Lengths and Intensities of the Maxima

(ethanol)			
λ	log ε	λ	log ε
2540	4.68	3520	4.06
2640	4.74	3695	4.33
2760	4.42	3825	4.35
2870	4.62	3895	4.40
2995	4.73	4090	3.76

* m.p. = 165-167° (corr.).

Table V: Absorption Spectrum of 4,9-Dimethyl-5,6-Benzthiophanthrene *; Wave Lengths and Intensities of the Maxima

(ethanol)			
λ	log ε	λ	log ε
2705	4.71	3455	4.15
2790	4.78	3570	3.76
2890	4.71	3770	3.44
3305	4.08		

* m.p. = 158-159° (corr.).

Effect of Alkyl and Alicyclic Substituents

Investigations, chiefly in the 1,2-benzanthracene series (16, 17, 22, 23), have shown that an alkyl group or an alicyclic ring (such as that present in cholanthrene) does not alter the shape of the absorption curve significantly nor does it change the molar extinction coefficients of most of the maxima very appreciably; alkyl and alicyclic derivatives of polynuclear aromatic

[2] Private communication from Dr. C. E. Dunlap.

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hydrocarbons can be related structurally to the parent unsubstituted hydrocarbon by comparison of the absorption spectra (12-14). Certain of the maxima may, however, be changed quite considerably in intensity by the introduction of an alkyl substituent and it would seem inadvisable to attempt to analyze quantitatively solutions of alkyl or alicyclic derivatives of these hydrocarbons unless the absorption curve of the particular alkyl or alicyclic derivative is available for comparison.

Cholanthrene, 20-methylcholanthrene, 2-methyl-3,4-benzpyrene, and 9,10-dimethyl-1,2-benzanthracene are the most potent carcinogenic hydrocarbons of this type and the spectra of all of these have been recorded (Table I).

Quantitative Analysis of Solutions of Pure Hydrocarbons

The accuracy with which pure hydrocarbons can be determined is limited by the accuracy with which the extinction coefficient can be determined, which, in turn, is limited by the accuracy of measurement of the density (log I_0/I). This has been discussed for photographic methods using a sector or Hilger-Spekker photometer by Holiday (15) who observes that "it may therefore be conservatively stated that the error in the visual match point method of spectrography is less than ± 0.055 E for a single matching" (E is here equivalent to the density). Twyman and Allsopp (26) consider that the *least* error in density measurement likely to be achieved in practice is ± 0.005 in the density reading. It is evident that as percentage errors these will be less at high densities than at low ones and quantitative measurements should always be made at densities greater than unity. It is not easy to match intensities nearer than 0.05 density units and at a density of 1.5 this corresponds to an error of ± 3.3 per cent. With photoelectric instruments greater accuracy in determining the density is possible but errors from other sources, such as uncertainty in cell length and cell alignment and effects of stray light, soon manifest themselves as factors limiting the significance of the measurements. Some experimental results obtained in these laboratories illustrating the accuracy obtainable in practice are shown in Table VI.

The limiting concentration which can be analyzed, assuming a solution of the pure hydrocarbon not contaminated with absorbing impurities, will be determined by the cell length and the molar extinction coefficient of the most intense maximum of the absorption curve. Of more interest than the limiting concentration is the limiting absolute quantity which can be determined and this will depend in addition on the ratio of the length to volume of the available cells. To fill the 1 cm. cell used in these laboratories, 2 ml. of solution are required while only 5.5 ml. are required for the 4 cm. cell; thus greater sensitivity is obtained by using a more dilute solution in the longer cell. Assuming that the concentration is based on a density measurement of 0.50 in a 4 cm. cell using 5.5 ml. of solution, the minimum quantities of several hydrocarbons which can be determined spectrographically are given in Table VII.

Analysis of Tissue Extracts

It is seldom that sensitivity or accuracy of the order discussed in the previous paragraph will be realized in the analysis of tissue extracts. Such extracts almost invariably contain other absorbing substances, in addition to the hydrocarbon, which give rise to "background" absorption; usually this is devoid of fine structure but often has a broad, flat maximum. The effect of this background absorption will depend upon the ratio of its intensity to that of the hydrocarbon and may vary from a negligible quantity to an amount sufficient to obscure the hydrocarbon curve completely. Examples of spectra showing various degrees of background absorption will be found in the succeeding papers (19, 20).

By suitable processing of the extracts, the background frequently may be reduced to a level which makes the analytical results significant. Lorenz and Shear (21) describe a method for the preparation of extracts of 1,2,5,6-dibenzanthracene from tumor tissue, based on a series of solvent partitions. Generally speaking,

Table VI: Spectrographic Analysis of Solutions of Pure Carcinogens and Related Substances

Substance	Added	Found	Error, in per cent
Anthracene	0.804γ	0.810γ	0.8
20-Methylcholanthrene	38.6	38.5	0.3
20-Methylcholanthrene	245	254	3.7
1,2-Benzanthryl-10-carbamidoacetic acid	67.5	65.5	3.0
	43.0	43.0	0.0
	39.8	40.8	2.5
	5.7	5.7	0.0
1,2-Benzanthryl-3-carbamidoacetic acid	79.0	82.6	4.5

Table VII: Minimum Quantities of Hydrocarbons Which Can Be Analyzed Spectrographically in a 4 cm. Cell Containing 5.5 ml. of Solution

Hydrocarbon	ε maximum	Quantity
1,2-Benzanthracene	89,130	1.75γ
1,2,5,6-Dibenzanthracene	173,800	1.09
3,4-Benzpyrene	60,260	2.90
20-Methylcholanthrene	89,130	2.07
4,9-Dimethyl-5,6-benzthiophanthrene	60,260	3.00

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methods of purification based upon chemical separation of the interfering substances from the hydrocarbon are preferable to methods based upon differences in physical properties. This may be illustrated in one case by the example given in Table VIII where the results of some experiments are recorded which were undertaken with the object of freeing extracts containing methylcholanthrene from hydroxyl-containing constituents. Two methods were investigated, a physical method based upon the preferential adsorption of the hydroxylic compounds on alumina from benzene solution and a chemical method based upon the reaction of the hydroxylic compounds with succinic anhydride to form an alkali-soluble half ester. While recovery of the 20-methylcholanthrene from the succinate reaction mixture was quantitative, an appreciable loss accompanied the chromatographic adsorption process.

Although complete removal of background absorption often may not be realizable, valuable data may still be obtained from spectrophotometric analyses of tissue extracts, provided it can be established that the relative contribution of the extraneous absorbing material is small compared with that of the hydrocarbon. A similar problem is encountered in the spectrographic analysis of vitamin A where, in spite of uncertainty introduced by this cause, the absorption intensity at 3,280 A. is still regarded as a more accurate indication of the vitamin A content of fish oils and similar products than colorimetric determination with the Carr Price reagent (27).

TABLE VIII: EFFECT OF PROCESSING ON THE SPECTROGRAPHIC ANALYSIS OF 20-METHYLCHOLANTHRENE

	Added	Found	Error, in per cent
Chromatographed from benzene on alumina	3.32 mgm.	3.08 mgm.	7.2
Refluxed with succinic anhydride in pyridine-dioxane	245γ	247γ	0.82
Refluxed with succinic anhydride in pyridine	245	245	0.0
Refluxed with maleic anhydride in ether	245	231	5.7

To illustrate the effects of background absorption, experiments may be cited in which rat or mouse liver tissue was ground with sand, and 3 to 4 mgm. of 1,2-benzanthracene added to one-half of the liver tissue, the remainder of the tissue being worked up as a control. After dehydration in a vacuum desiccator, the two samples were extracted in Soxhlet extractors with chloroform until the fresh extracts from the sample containing added hydrocarbon were no longer fluorescent (24 hrs.). After removal of the solvent *in vacuo* in a current of nitrogen the extracts were dissolved in 50 ml. of 2 N potassium hydroxide in 90 per cent methanol and refluxed for 2.5 hours. The saponification liquor was diluted with water, extracted with ether and the ether extracts, after removal of the solvent, dissolved in 50 ml. of ethanol, and the spectra determined, the intensity being calculated as $E^{1\%}_{1\text{ cm.}}$ based on the weight of the extract (Fig. 5). In this experiment the absorption spectrum of the control extract may be regarded as the background absorption of the extract containing added 1,2-benzanthracene and by subtracting this background curve a corrected value for the 1,2-benzanthracene content of the extract can be calculated. The results of two such experiments using mouse and rat livers, respectively, are summarized in Table IX. In one case where 3.34 mgm. of hydrocarbon were added to 2 gm. of dried ground rat liver tissue the quantity recovered was 3.58 mgm., neglecting the background absorption, which fell to 3.49 mgm. on making the correction. In this case the position of maximum background absorption at 3,220 A. and the fact that the extract gave a deep blue color with the Carr Price reagent suggested that vitamin A might be the substance contributing most to the background absorption. Treatment of the extract with maleic anhydride in ether removed the background absorption almost completely but also caused some loss of 1,2-benzanthracene. This is shown up in the spectrographic results obtained after treatment with maleic anhydride where the correction for the control is negligible but a significant fall in the estimated 1,2-benzanthracene also has occurred (Table IX).

TABLE IX: EFFECT OF BACKGROUND ABSORPTION ON THE SPECTROGRAPHIC ANALYSIS OF 1,2-BENZANTHRACENE IN RAT AND MOUSE LIVER TISSUE

	Found, mgm.
Experiment 1. 3.34 mgm. added to 2 gm. rat liver	
Uncorrected for background absorption	3.58
Corrected by subtraction of background determined on control experiment	3.49
Same extract after treatment with maleic anhydride in ether (to remove vitamin A)	3.08
Same, corrected for background absorption by control experiment	3.05
Experiment 2. 4.70 mgm. added to 1.58 gm. mouse liver	
Uncorrected for background absorption	4.79
Corrected	4.56

This method of running control spectra is probably the most satisfactory way of correcting for the effect of background absorption; unfortunately, in addition to the fact that it involves a complete duplication of the experimental procedure and is therefore time-consuming, it may not always be reliable since it cannot be known with certainty that the injection of the carcinogen may not bring about secondary changes in metabolism which alter the concentration of the sub-

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stances in the extracts which are responsible for the background absorption.

Graphical Estimation of Effects of Background Absorption

An indication of the contribution of the background to the total absorption may be obtained by comparing the shape of the experimentally observed curve with that of the pure hydrocarbon. It is not common to find background absorption of a uniform intensity over the whole spectrum and comparison of the experimental curve with a "master curve" of the hydrocarbon drawn on the same scale [3] will indicate the region in which the background is a minimum as that where the two curves fit most closely; the extent of the deviation of the experimental curve from the master curve will enable a qualitative estimate to be made of the degree to which the extraneous absorption is influencing the spectrum. By calculating the concentration from extinction coefficients in the region of the curve which agrees most closely with the master curve, the effect of the background absorption can be reduced to a minimum. This is illustrated in Fig. 6 where the curve for 1,2-benzanthracene, plotted on the same scale, is superimposed on the experimental curve for a liver extract containing 1,2-benzanthracene. The presence of a maximum in the background absorption near 3,200 A. is evident from the deviation between the two curves in this region. The master curve, plotted on the same scale on transparent paper is placed over the experimental curve and the ordinates adjusted so that it touches the experimental curve where possible, *but at no point lies above it.* At any wave length, the total absorption in the experimental curve will be due to two components, the hydrocarbon (E_h) and the background component (E_b). By this graphical procedure the curves will coincide where E_b is a minimum and, at all wave lengths where E_b is greater, the experimental curve will be more intense, the difference between the curves giving a measure of the magnitude of the background effect. By calculating the concentration of the hydrocarbon from points where the experimental and master curves coincide, the effect of the background is reduced to a minimum. The concentration of hydrocarbon so calculated may be regarded normally as a maximum and the significance which can be attached to the result will depend upon the extent to which the experimental curve deviates from the master curve. Where the two coincide over the greater part of the spectrum, the calculated concentration can be accepted with confidence as accurate. Where the curves differ markedly the results may be in error, as in such cases it is probable that even in the region where there is coincidence considerable enhancement of the intensity due to background may be occurring and the concentration of hydrocarbon so calculated is likely to be high.[4]

[3] As this is purely an empirical method, curves plotted on a logarithmic intensity scale are convenient.

Summary

The principles of ultraviolet absorption spectrophotometry are discussed with reference to the application of spectrographic methods to the quantitative analysis of carcinogenic hydrocarbons and their metabolic products in tissue extracts. The data available concerning the absorption spectra of carcinogenic hydrocarbons and related substances are reviewed and the factors limiting the accuracy and sensitivity of the method are considered. In tissue extracts the presence of background absorption due to other absorbing constituents is a major factor in limiting the value of spectrographic analysis of such extracts and a rapid method of estimating approximately the degree to which such background interference is present in any given case is presented.

The author wishes to express his appreciation of the interest and encouragement of Professor L. F. Fieser and to thank Mr. John Clarke and Mrs. J. V. Burkhead for technical assistance.

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[4] In the presence of continuous background absorption the resolution of the fine structure absorption bands is decreased and the maxima may be suppressed. This effect will counteract the increase in intensity due to the additive effect of the background. Other factors may also be present in some extracts which affect the intensity. If the solutions are not optically clear there will be a loss of light from scattering by suspended particles which will enhance the apparent absorption. This effect will be very much greater at shorter wave lengths since, according to the Rayleigh equation, the intensity of the scattered light is a linear function of the inverse fourth power of the wave length.

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The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. I. Introduction

R. Norman Jones

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