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THE METABOLIC DEGRADATION IN THE MOUSE OF DIBENZANTHRACENE LABELED IN THE 9 AND 10 POSITIONS WITH CARBON 14

by

Charles Heidelberger, Martha R. Kirk, and Marion S. Perkins

January 30, 1948

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ABSTRACT

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Charles Heidelberger, Martha R. Kirk, and Marion S. Perkins

From the Department of Chemistry,
and the Radiation Laboratory,
University of California, Berkeley.

30 January 1948

ABSTRACT

Evidence has been presented which proves that dibenzanthracene is metabolized by the mouse into at least four substances, and some speculations as to the sites of this degradation have been advanced.

This paper is based upon work performed under Contract No. W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley.

To be published in Cancer

THE METABOLIC DEGRADATION IN THE MOUSE OF DIBENZANTHRACENE LABELED IN THE 9 AND 10 POSITIONS WITH CARBON 14

by
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From the Department of Chemistry,
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30 January 1948

In the accompanying paper (2) studies of the distribution of radioactivity following the administration of dibenzanthracene labeled in the 9 and 10 positions with carbon fourteen have been described. In contrast to all earlier reports, a virtually quantitative recovery of the dose administered has been accomplished. This is due to the fact that all substances derived from the carcinogen and still containing the labeled carbon atoms may be traced by following the radioactivity, whereas the spectroscopic or fluorometric methods of analysis previously used are capable of directly detecting only those metabolites which maintain the intact pentacyclic aromatic ring system. This non-quantitative

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1. This paper is based on work performed under Contract #W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, California. Preliminary reports of this work were presented at the meeting of the American Association for Cancer Research, Inc. in Chicago, May, 1947, at the Fourth International Cancer Congress, St. Louis, Sept., 1947, and at the 50th anniversary meeting of the Columbus Section of the American Chemical Society, October, 1947. Abstracts of these talks have been submitted as Reports BP-86 and BC-72 and have been declassified.

2. C. Heidelberger and H. B. Jones, UCRL-43.

recovery of carcinogen, and apparent disappearance have always been interpreted (3) as being due to metabolic degradation of the compound to other substances with non-characteristic absorption spectra or fluorescence. Levi and Boyland (4) in 1937 reported the isolation of a dihydroxydibenzanthracene from rabbit urine, and Dobriner, Rhoads and Lavin (5), in an extremely thorough study of the excreta of various animals given dibenzanthracene subcutaneously, isolated another compound from the urine and feces of rats and mice, which was proved to be 4',8'-dihydroxydibenzanthracene. In spite of a very extensive fractionation of excreta, bile, intestinal contents and whole mice, these investigators were unable to detect any absorption spectra that appeared to be derived from the administered dibenzanthracene. Yet only a small percentage of the original material could be accounted for.

It was decided to reinvestigate the problem of metabolic degradation using the radioactive carcinogen. Since it has been shown (2) that most of the radioactivity is found in the excreta, a systematic study was undertaken in order to gain some information as to the types of substances produced as degradation products, and to contrast and compare the patterns of metabolism of the dibenzanthracene when administered by different routes. Accordingly, we have submitted to chemical fractionation urines, feces, biles, subcutaneous injection sites, and tumors produced by the labeled carcinogen. In these fractionations the radioactivity was determined in each step, and it was shown immediately that the compound is degraded to several substances, some highly

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3. a) I. Berenblum and L. P. Kendall, Biochem. J. 30, 429 (1936).
   b) J. G. Chalmers and P. R. Peacock, Biochem. J. 30, 1424 (1936).
   c) R. N. Jones, C. E. Dunlap, and C. J. Gogek, Cancer Research 4, 209 (1944).

4. A. A. Levi and E. Boyland, Chem. and Industry 15, 446 (1937).

5. K. Dobriner, C. P. Rhoads, and G. I. Lavin, Cancer Research 2, 95 (1942).

water-soluble, which are chemically very different from the original material, and must involve some deep-seated changes in the molecule. It is not surprising then, that spectroscopic methods failed to detect these substances, for even if new bands were observed there could be no definite demonstration that these originated from metabolites of dibenzanthracene.

Water-soluble derivatives of dibenzanthracene have been reported as having been obtained by photochemical oxidation of the compound in benzene solution. Boyland and Boyland (6) in 1934 studied the effect of these substances on various enzyme systems and Alsopp and Szigeti (7) have determined their absorption spectra and tested them for carcinogenicity. We have found that the products of photooxidation of dibenzanthracene are different from the water-soluble materials produced by metabolism in the mouse.

Experimental Methods

In order to compare the type and extent of metabolic degradation of dibenzanthracene following various modes of administration to mice, these samples have been collected: feces, urine, and bile from animals given the carcinogen intravenously as an aqueous colloid and intraperitoneally in tricaprylin; feces and urine after stomach tube administration in tricaprylin; feces and injection sites following subcutaneous injection in tricaprylin; and tumors induced by the labeled dibenzanthracene. The bile samples were collected through one day, and the total urine and feces, three days after administration. These specimens were then subjected to the extraction procedure shown in Table I.

The feces were dried at 70° in vacuum, were ground to a fine powder, and the radioactivity was determined after combusion of an aliquot as de-

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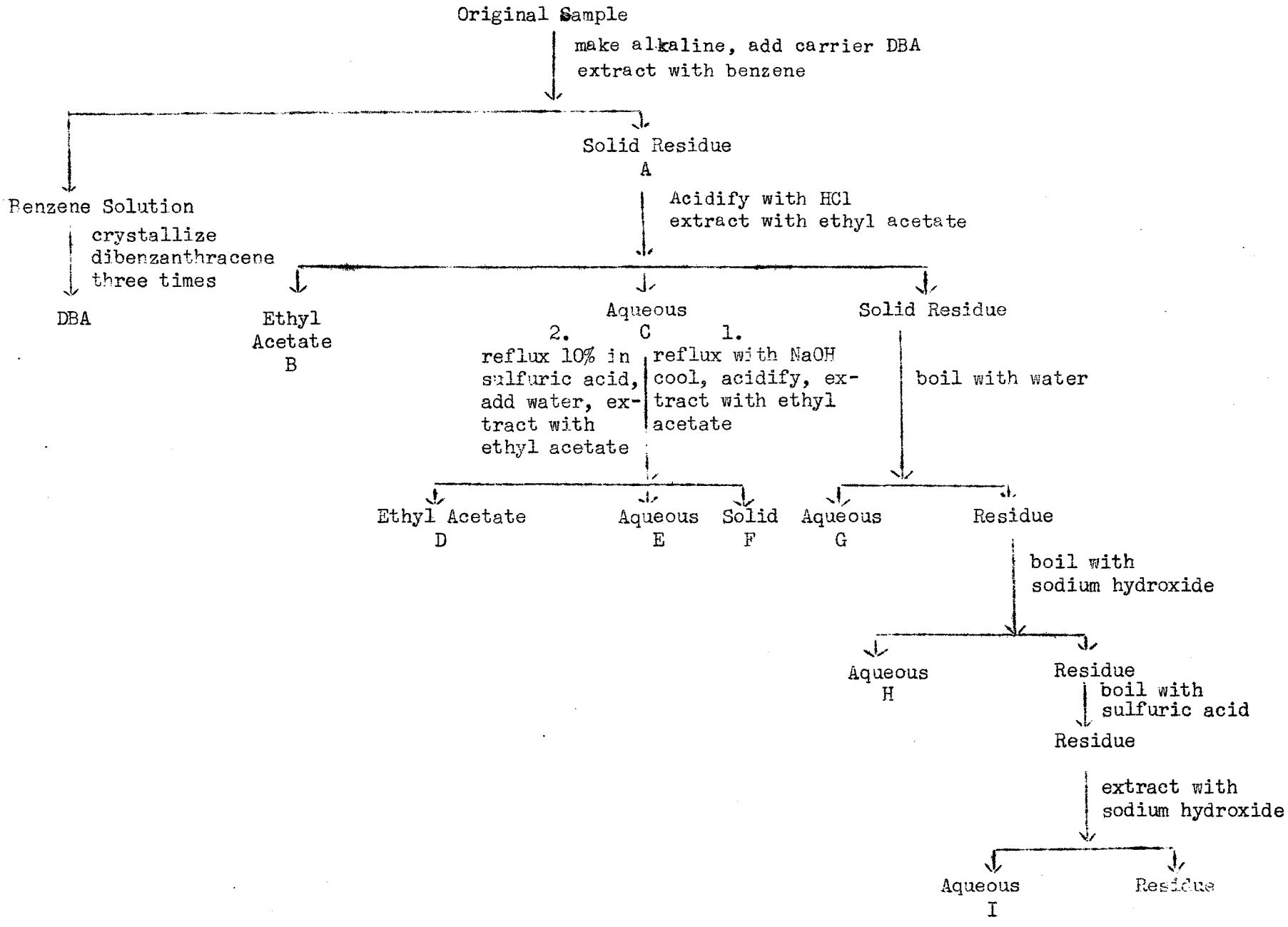
6. E. Boyland and M. E. Boyland, Biochem. J. 28, 244 (1934).

7. C. B. Alsopp and B. Szigeti, Cancer Research 6, 14 (1946).

TABLE I


Original Sample
make alkaline, add carrier DBA
extract with benzene
Benzene Solution
crystallize
dibenzanthracene
three times
DBA
Solid Residue
A
Acidify with HCl
extract with ethyl acetate
Ethyl
Acetate
B
Aqueous
C
2.
reflux 10% in
sulfuric acid,
add water, ex-
tract with
ethyl acetate
1.
reflux with NaOH
cool, acidify, ex-
tract with ethyl
acetate
Solid Residue
boil with water
Ethyl Acetate
D
Aqueous
E
Solid
F
Aqueous
G
Residue
boil with
sodium hydroxide
Aqueous
H
Residue
boil with
sulfuric acid
Residue
extract with
sodium hydroxide
Aqueous
I
Residue

scribed previously (2). An aliquot of the urines was plated directly, a little dilute sodium hydroxide was added to the remainder, and the water was removed as the azeotrope during the prolonged benzene extraction. Biles were evaporated to dryness after an aliquot was plated directly and a trace of alkali added. Injection sites and tumors were homogenized, and the activity was determined by combustion of a sample. A little alkali was added, and the water was removed as the azeotrope during the benzene extraction.

The liquid-solid extractions were carried out in small flasks fitted with reflux condensers; the liquid-liquid extractions were performed entirely in centrifuge tubes, the upper layer being removed with a pipette. The radioactivity of both fractions was determined in all cases, so that a constant check was maintained on total quantities, and losses might be spotted immediately. Moreover, this procedure furnished partition coefficients for each step, information very valuable for attempts at complete chemical identifications.

The radioactivities of solid samples were determined after combustions, as described previously (2). Liquid samples were plated directly (8) and counted with a thin window Geiger Mueller Counter. The empty plate is mounted on a rotating turntable with a blast of hot air from a hair dryer directed upon it. An aliquot of the solution to be measured is slowly expelled by means of a syringe, from a micro-pipette onto the rotating plate in such a way that the solvent evaporates immediately and the residue is spread evenly on the plate. Because the weight of sample on the plate seldom exceeds a few tenths of a milligram, the self absorption of the β-particle may be neglected. The accuracy of this method is not as great as that obtained when barium carbonate is mounted, but the advantages of speed, convenience, and

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8. This technique was devised by Dr. A. A. Benson of these laboratories.

the fact that the sample is not destroyed make this technique ideal for the type of work described here. The total experimental error on each sample does not exceed 10%, and this is substantiated by the excellent recoveries in the extraction experiments. This method also makes it possible to gain considerable information as to the organic chemistry of labeled compounds on the microgram scale. It is, of course, unsuitable for the assay of volatile compounds.

A summary of the results obtained in the extraction experiments is shown in Table II. These are expressed as counts per minute and as percentage of the radioactivity in the original sample. The total counts recovered are also expressed as the number of milligrams of dibenzanthracene to which the radioactivity would be equivalent. It is quite evident from casual inspection of these data that dibenzanthracene is very extensively metabolized by the mouse to other substances differing widely in chemical characteristics from the original carcinogen. However, in order to gain more detailed information as to the chemistry of the various fractions, each was subjected to further examination.

Dibenzanthracene

The dibenzanthracene is determined by means of carrier technique. A known amount of non-labeled dibenzanthracene is added during the fractionation and is crystallized from the benzene solution. It is purified by two recrystallizations, one from acetic acid, and the other from benzene. The dibenzanthracene is combusted and its radioactivity determined. Under these conditions this radioactivity could only originate from the labeled carcinogen, and this furnishes an extremely accurate and sensitive method for the quantitative determination of non-metabolized, labeled dibenzanthracene on the micro-

TABLE II

	Tot. cts. recovered (mg. of DBA)	DBA (♂)	A	B	C	D	E	F	G	H	I	% Recovery
Intravenous: Feces	12,900 (0.193)	730 11 ♂ 5.6%		1870 14%	2190 17%	1 127 5.8%	1 1900 15%	1 0	2050 16%	4770 37%	760 5.9%	56% (spill in 1st step)
Intravenous: Urine	1180 (0.018)	47 0.7 ♂ 4.0%		170 15%	610 52%	1 0	1 354 30%	1 113 19%	32 2.7%	300 26%	0	85%
Intravenous: Bile	2600 (0.039)	600 9.0 ♂ 23%		20 0.7%	1900 73%	1 60 2%	1 1800 69%	1 0	80 3.0%	0	0	90%
Intraperitoneal: Feces	11,000 (0.164)	130 1.9 ♂ 1.2%		460 4.2%	80 0.7%				3500 32%	3000 27%	1400 13%	87%
Intraperitoneal: Urine	1780 (0.027)	35 0.53 ♂ 2.0%		490 24%	870 48%	1 87 4.8%	1 680 37%	1 156 8.5%	18 1.0%	450 25%	0	103%
Intraperitoneal: Bile	880 (0.013)	50 0.75 ♂ 7%		140 20%	460 64%	1 110 16%	1 410 58%	1 36 5%	0	30 4%	0	81%
Stomach tube: feces	8620 (0.130)	3900 58 ♂ 45%		510 6%	810 9%	1 180 2.1%	1 450 5.2%	1 60 0.7%	90 1.0%	2700 31%	460 5.3%	86%
Stomach tube: urine	3360 (0.050)	150 2.2 ♂ 4.5%		620 18%	1800 54%	1 70 2%	1 670 20%	1 840 25%	100 3%	690 20%	0	90%
Subcutaneous: Feces	2820 (0.047)	305 4.5 ♂ 11%		400 14%	200 7%	2 110 3.9%	2 110 3.9%	2 285 10%	345 12%	1100 39%	465 17%	93%

TABLE II (continued)

	Tot. cts. recovered (mg. of DBA)	DBA %	A	B	C	D	E	F	G	H	I	% Recovery
Subcutaneous Injection Site, 1 wk.	45,300 (0.675)	44,100 660 97%	730 1.5%									100%
Subcutaneous Injection Site, 3 mos.	4160 (0.062)	4020 60 96.5%	135 3.5%									100%
Tumor: 6 mos., tricaprylin, hyperkeratosis of skin with early spindle-cell sarcoma	460 (0.0069)	460 6.9 100%										100%
Tumor: 10 mos., mouse fat spindle-cell sarcoma	577 (0.0086)	310 4.6 54%		90 13%	127 22%				0	50 8.7%	0	84%
Tumor: 6 mos., mouse fat spindle-cell sarcoma	1050 (0.016)	160 2.4 15%		0	240 23%	1 72 0.6%	1 216 21%	1 0	330 32%	270 25%	0	79%
Tumor: 6 mos., tricaprylin, spindle-cell sarcoma	4030 (0.060)	3700 55 92%		0	29 0.7%				113 2.8%	190 4.7%	0	98%
Tumor: 8 mos., tricaprylin, spindle-cell sarcoma with marked hyalinization	4200 (0.063)	4130 62 99%	0									99%
Photo-oxidation, 1 hr. in benzene solution	40,500 (0.605)	29,700 450 73%							($NaHCO_3$) 7500 18.5%	2900 7.2%	0	100%

gram scale.

The benzene solution from which the dibenzanthracene is crystallized contains no other compounds containing labeled carbon, because activity measurements on this solution always correspond closely to the activity of the total dibenzanthracene.

Fraction B

This fraction contains organic material that is probably unconjugated, since it is obtained by very mild treatment. This is also the fraction that would contain dihydroxydibenzanthracene if not conjugated. Unfortunately, none of this compound was available for use as carrier. Table III represents an attempt to gain further information as to the chemical components of this fraction. It shows that there is acidic material present that is extracted by bicarbonate. However, further extraction of the organic phase with alkali does not remove more activity, indicating that no phenolic compounds are present in this mixture. Furthermore, it was found that there was a very considerable amount of activity present in this fraction that is not extracted with acid or alkali. This neutral material is not dibenzanthracene, for no radioactivity was found in carrier dibenzanthracene added to the solution. This material is evidently some neutral metabolite that was not taken up in the original benzene extraction.

In order to detect dihydroxydibenzanthracene with more certainty, the experiment shown in Table IV was carried out. Fraction B was acetylated under the conditions described by Cason and Fieser (9), and the mixture was made alkaline and extracted with ethyl acetate. The activity remaining in the aqueous is derived from acidic material. The ethyl acetate layer was hydrolyzed

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9. J. Cason and L. F. Fieser, J. Am. Chem. Soc. 62, 2681 (1940).

TABLE III

Fraction B, Feces

from Stomach Tube

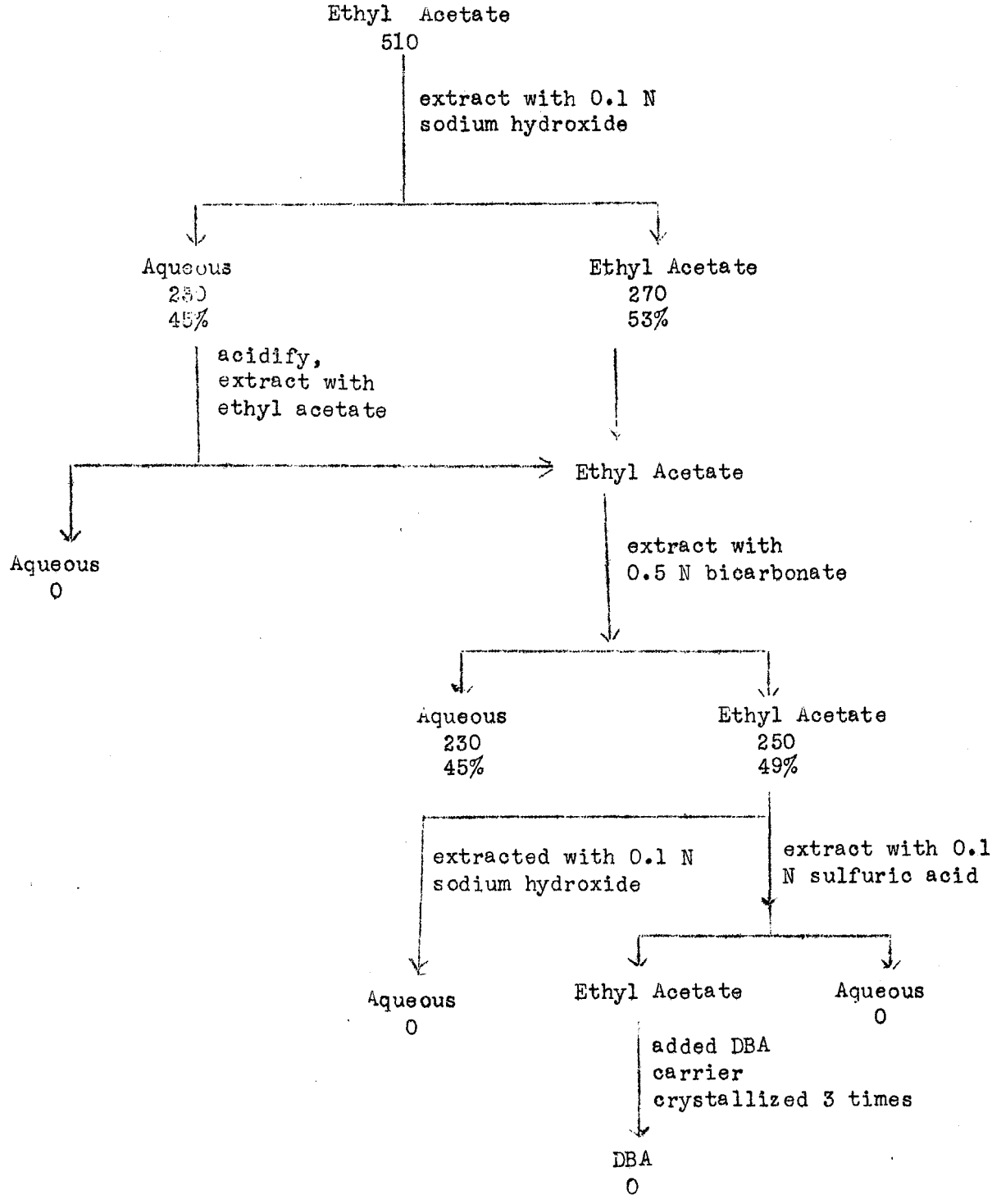
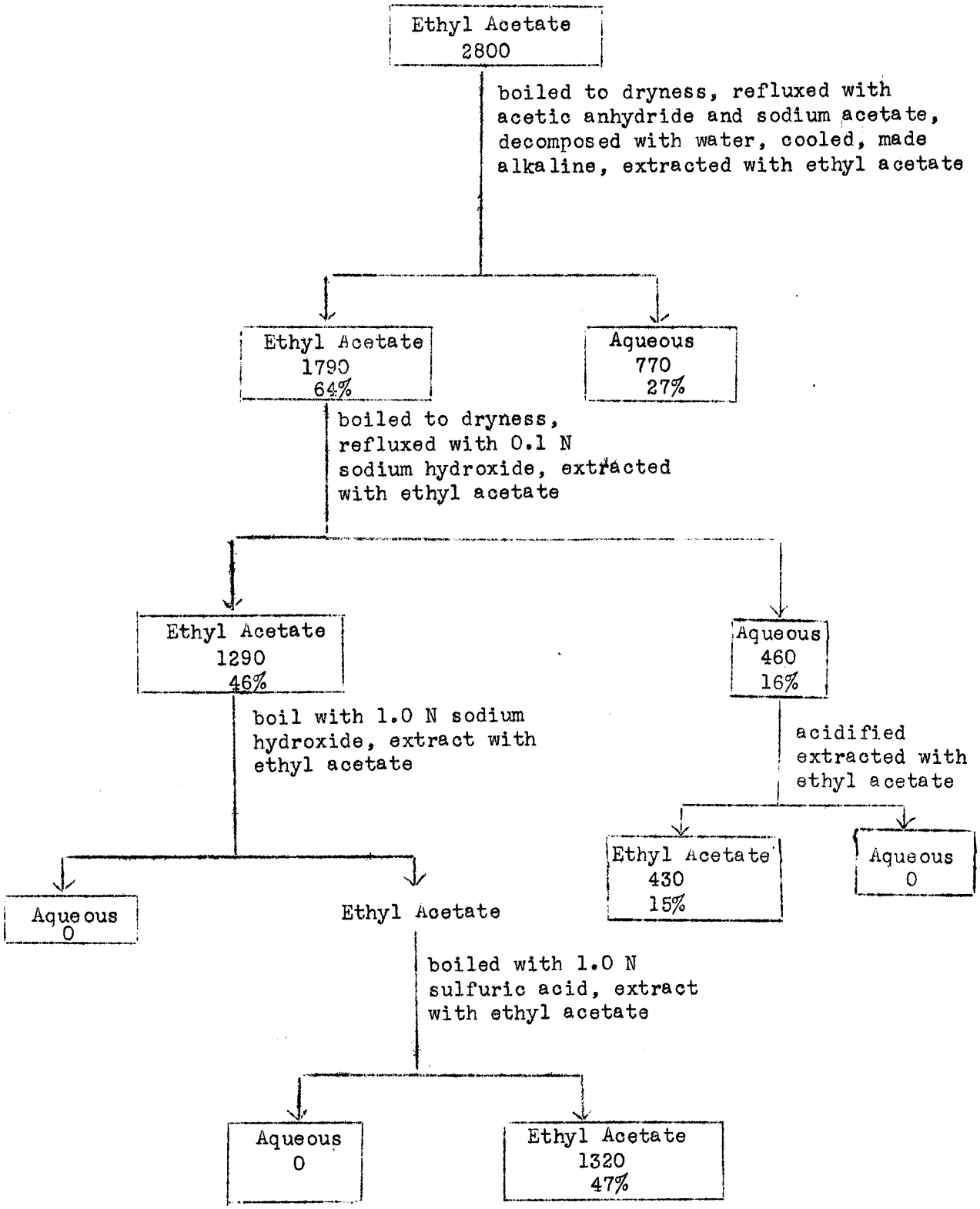


TABLE IV

Fraction B

Feces from Stomach Tube



with alkali and re-extracted with ethyl acetate. There was radioactivity in the alkali this time, which after acidification was taken up in the organic solvent. This represents phenolic material, and is probably dihydroxydibenzanthracene, although there is no further evidence on this point. This fraction only accounts for 15% of the radioactivity in Fraction B or 2.7% of the activity in the entire feces sample. Again a considerable amount of neutral material was found, which was not affected by drastic acid and alkaline hydrolysis.

Fraction C

This fraction appears to consist of some extremely water soluble organic substances together with some less soluble material that is conjugated with water-solubilizing groups. Fraction C is split by hydrolysis into fractions D, E, and F. Procedure 1 is an alkaline hydrolysis, 2, an acid hydrolysis, as indicated in Tables I and II. Fraction D contains the substances that were conjugated with solubilizing groups, and is acidic in nature, for most of the activity is extracted from the ethyl acetate by alkaline solution. The radioactivity of Fraction E is in truly water-soluble form, even after rather drastic hydrolytic treatment. This activity is not extracted by ethyl acetate from the aqueous phase at acidic, basic pH's or at neutrality. Fraction F is a solid that appears after hydrolysis and carries considerable activity. The solid must be a component of the urine or feces, since several milligrams of material are invariably obtained whereas the radioactivity it carries generally corresponds to only a few micrograms of compound. The solid is usually soluble in alkali, as is the radioactivity, but this varies from sample to sample, and little progress has been made as yet in the separation of the radioactive compounds from this solid carrier. Thus, the chemical nature of this fraction is not clearly known, although it appears to be acidic, and yet

is not extracted by organic solvents.

Fraction G

This fraction is obtained by boiling with water the solid residue from the original sample following the removal of fractions B and C. This, like E, consists of truly water soluble material together with some substance that is rendered soluble by conjugation. Further processing of this fraction is shown in Table V, and similar results were obtained from several other samples. Again troublesome solids were encountered, and have not been further characterized. These results indicate that the ethyl acetate fraction is very similar to the acidic material of Fraction B. If the organic compound containing the labeled carbon is the same, however, it is present in conjugated form in Fraction G, since it had been made water-soluble. It also appears that the water-soluble fraction after hydrolysis is the same as Fraction E. These points of similarity cannot be completely confirmed until the final identification of the compounds in these fractions has been accomplished.

Fraction H

This fraction is obtained by boiling with alkali the solid residue from Fraction G, and contains acidic and water-soluble substances that are released by alkaline hydrolysis and were probably conjugated with proteins and other solid material. Further processing of this sample is shown in Table VI, and these results correspond closely with those obtained by similar treatment of other samples. Again, ethyl acetate does not extract any radioactivity from water-soluble fraction whether the solution is basic, acidic, or neutral. The distribution of activity of the alkaline extract of the ethyl acetate solution suggests that this fraction might contain acidic material very similar to that found in other ethyl acetate fractions, such as B and D. The precipitate that

Table V

Fraction G

Feces from Intravenous Injection

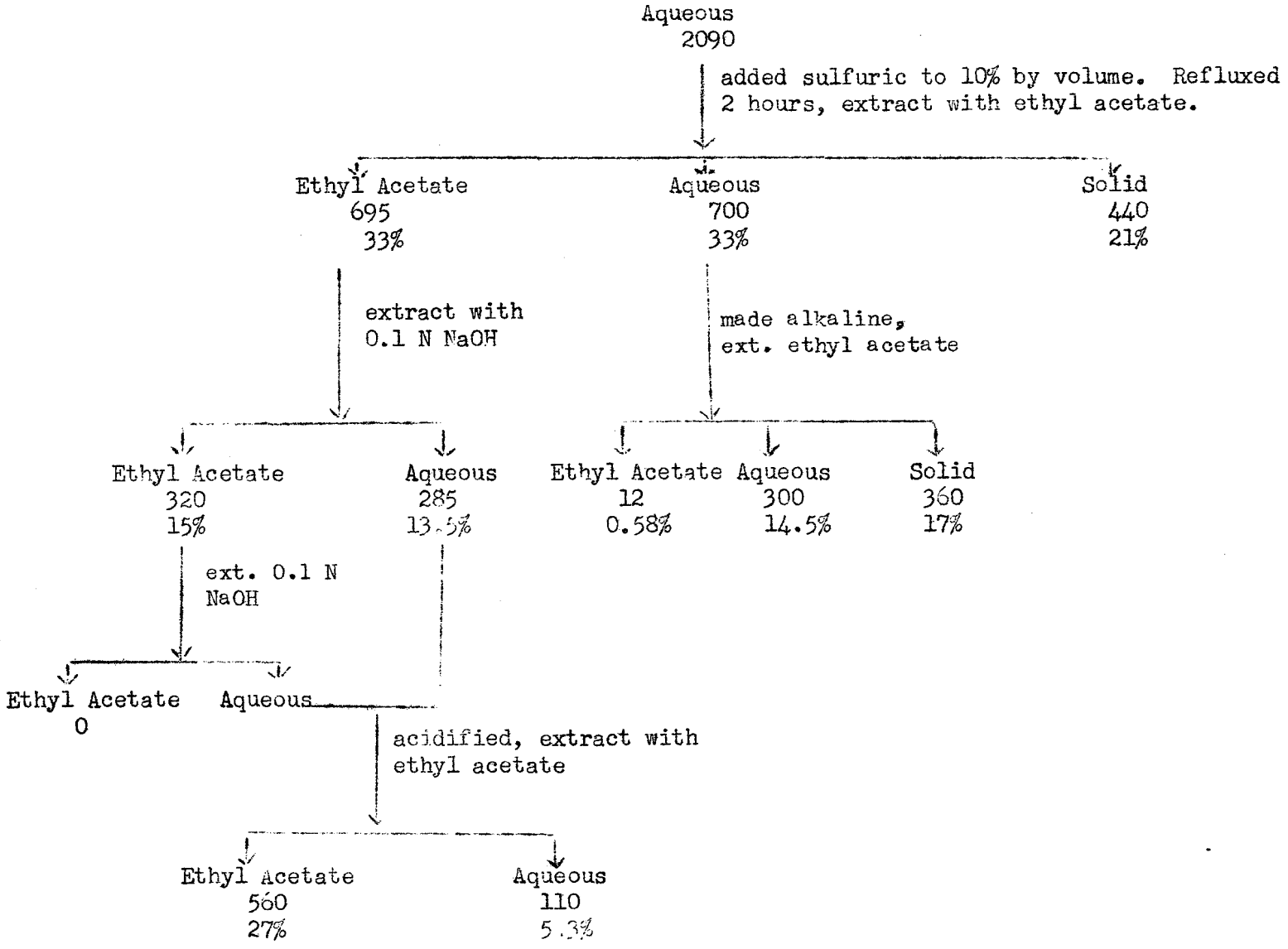
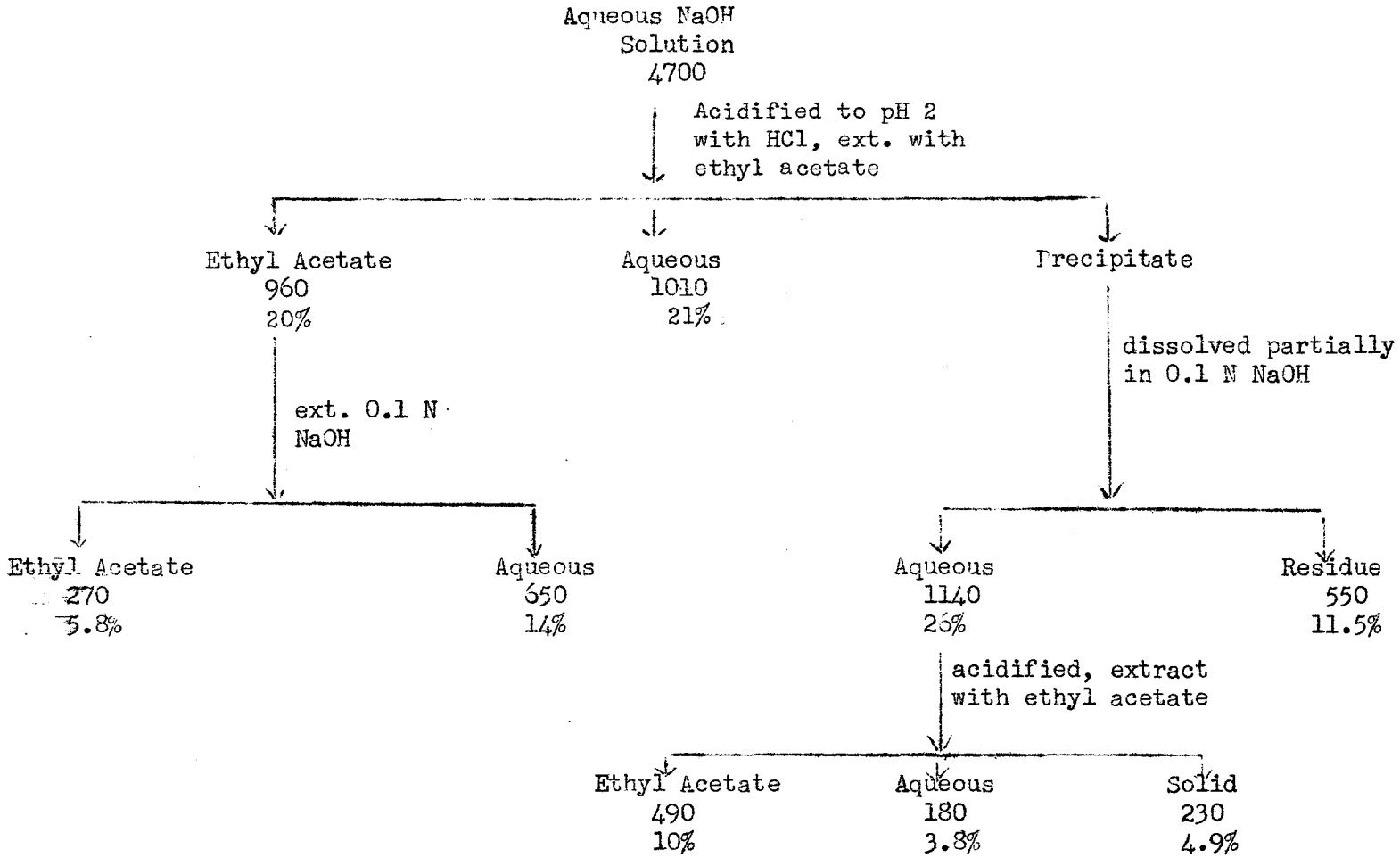


Table VI
Fraction H
Feces from Intravenous Colloid



formed on acidification of the original solution partially dissolved in alkali, and on acidification of an appreciable amount of radioactivity was extracted by ethyl acetate.

Fraction I

In some samples there was still radioactivity in the residue from the alkaline extraction, Fraction H. When this residue was refluxed with dilute sulfuric acid, no activity appeared in the aqueous phase. However, when the residue was then extracted with alkali, radioactivity appeared in the aqueous solution. This activity represents acidic compounds that are possibly conjugated with protein or other material in such a way that they are hydrolyzed by acid and not by base.

Table VII

At the kind suggestion of Dr. James Cason, who called our attention to the fact that dihydroxydibenzanthracene is very unstable in acidic solution, the experiment shown in Table VII was carried out in order to determine whether some of the "metabolites" encountered in the previously described work might have arisen from the phenolic compound as an artifact during the isolation procedure.

The feces sample was acetylated before further processing in order to protect any dihydroxydibenzanthracene that might be present, and the mixture was extracted with ethyl acetate, Fraction 1. This was then hydrolyzed with alkali, and re-extracted with ethyl acetate. Carrier dibenzanthracene was added to this fraction and after purification, it was found that 6.5% of the activity was due to the unchanged carcinogen. This represents 1.6 δ of compound. Fraction 2 was acidified and extracted with ethyl acetate, and Fraction 3 contains phenolic material, probably dihydroxydibenzanthracene.

TABLE VII (part 1)

Feces Following Intraperitoneal Injection
1640 (0.025 mg. DBA)

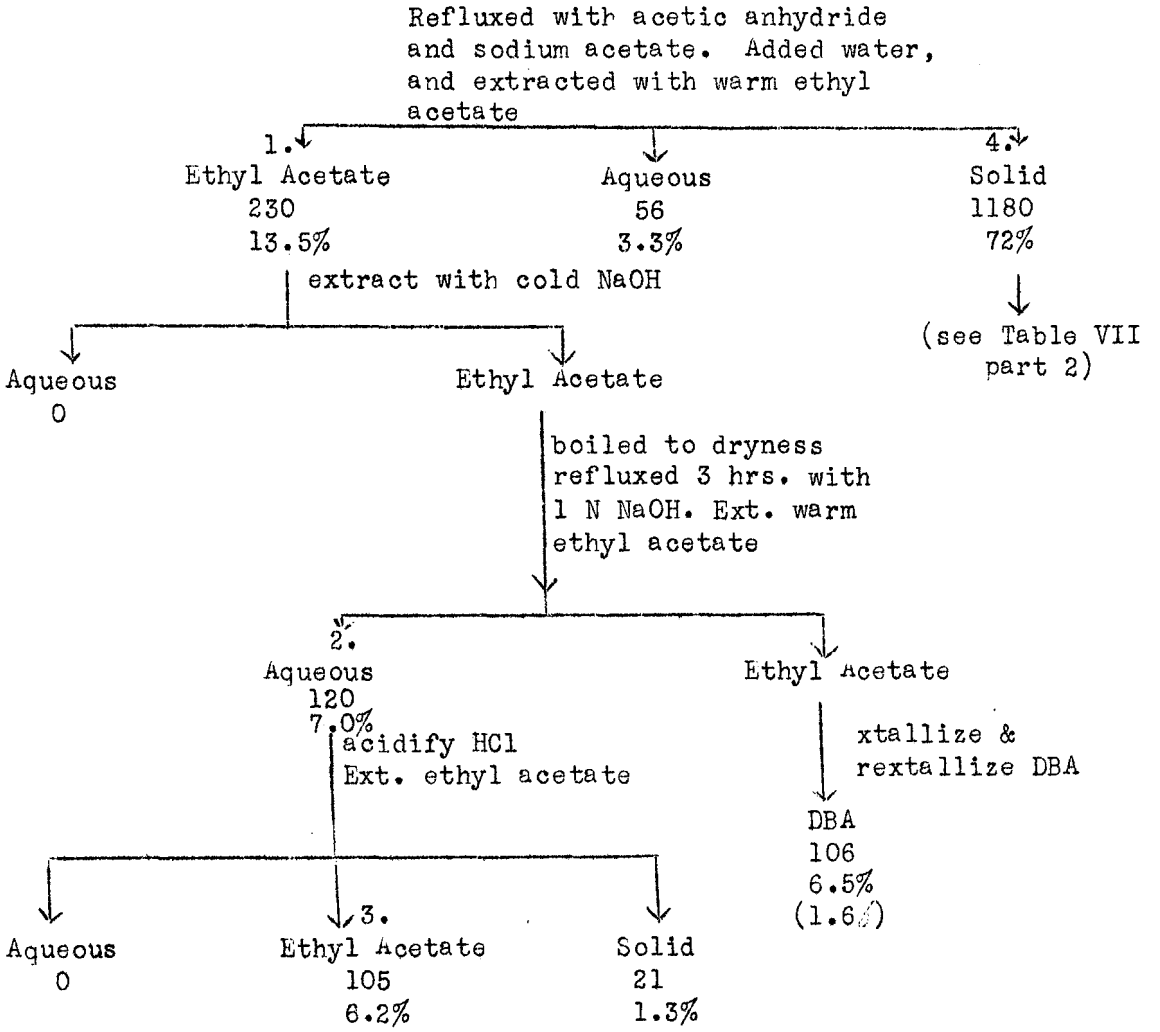
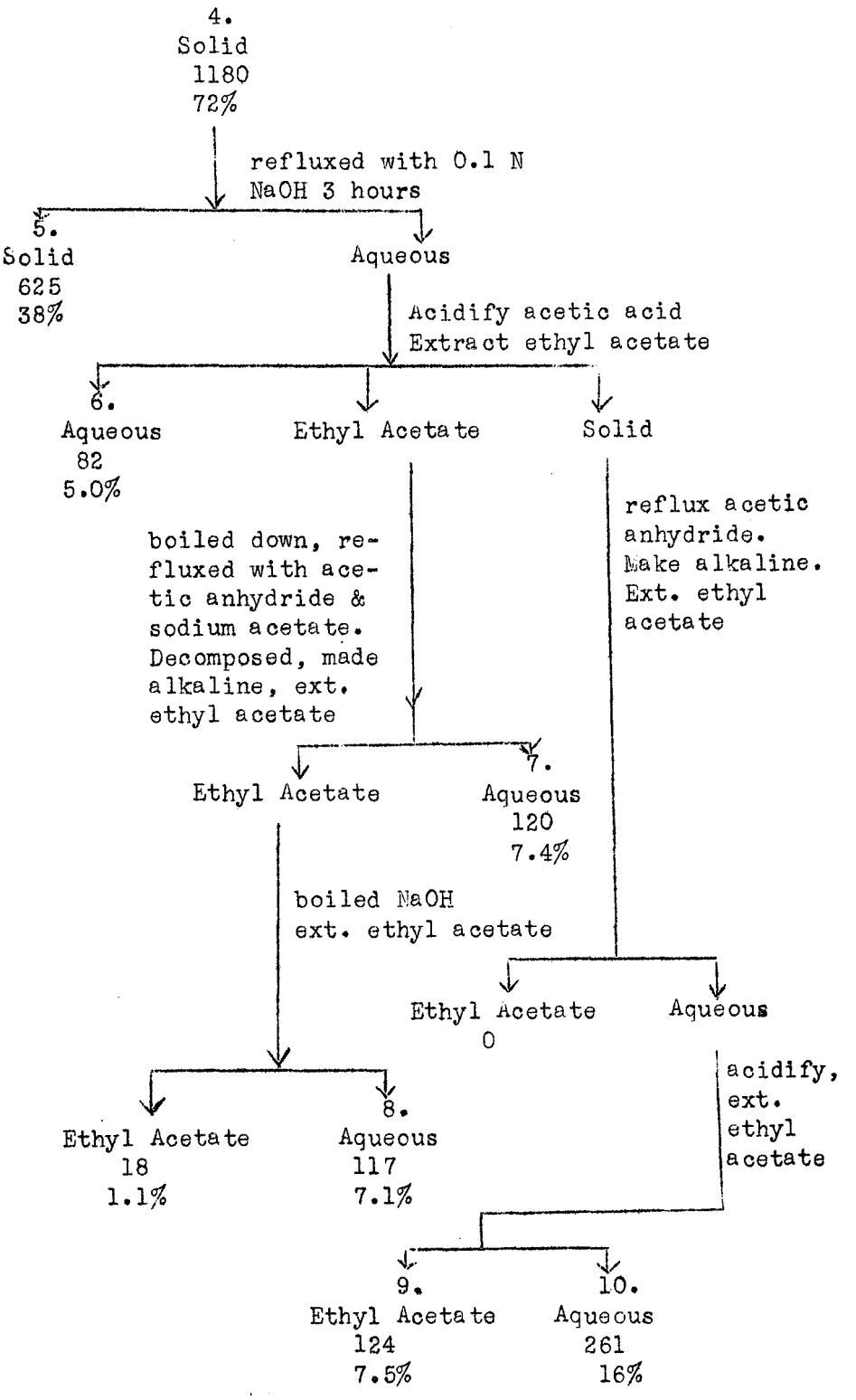


TABLE VII (part 2)



The solid precipitate, Fraction 4 was refluxed with alkali, and the residual solid, Fraction 5 was not further investigated. The aqueous solution was acidified, extracted with ethyl acetate, and re-acetylated. Further processing resulted in various fractions of which number 8 is phenolic in nature. Thus, Fractions 3 and 8, which account for almost 14% of the radioactivity in the entire specimen, are phenolic in nature; Fractions 7, 9, and 10 contain 31% of the activity, and are water-soluble. Fraction 5 was not further investigated, but in the light of other experiments, it very likely consists of water-soluble substances in conjugated form. This appreciable amount of phenolic material is greater than has been previously observed in other investigations and in our other experiments, and indicates that some of the degradation products may have arisen from a phenolic compound (probably dihydroxydibenzanthracene) during the isolation procedure.

Metabolic Degradation Following Different Modes of Administration

Intravenous

It is apparent from Table II that dibenzanthracene administerered by intravenous injection of the aqueous colloid is very extensively degraded, since only 5.6% and 4.0% of the radioactivity in the feces and urine respectively is due to dibenzanthracene itself. These activities correspond to 11 and 0.7 % of the carcinogen. It is clear from inspection of data for the bile, that the liver is one of the sites of degradation, because there is only 26% of dibenzanthracene radioactivity in the sample and it has already been shown (2) that there is immediate uptake of activity by the liver which then empties it into the bile. Since it has also been demonstrated that the only source of activity in the intestinal contents is derived from the bile, it is evident that further degradation takes place in the intestinal tract, because of the considerably

smaller quantity of non-metabolized carcinogen in the feces than in the bile. It is rather surprising that such a large and insoluble molecule could be eliminated through the kidneys and appear in the urine, but similar observations have been made by spectroscopic methods (3c). It is possible, however, that this may have arisen due to contamination of the urine by the feces, since it was impossible under the conditions of our experiments to prevent these excreta from coming into contact with each other. The B fraction contains considerably more activity in the urine and feces than does the bile. The absence of an H fraction in the bile probably indicates only that the metabolite does not conjugate with the components of the bile.

Stomach Tube

There is a considerably larger amount of unchanged dibenzanthracene in the feces (45%) by this route than by the others, but only 4.5% of the radioactivity in the urine sample can be accounted for as dibenzanthracene. This probably indicates that the intestinal tract is not as efficient as the liver in metabolizing the compound. The majority of the water soluble material in the feces is bound in some way (Fraction H), whereas in the urine most of the activity appears in Fraction C and is in a less conjugated state. However, the degradation in these cases must take place exclusively in the gastrointestinal tract.

Intraperitoneal

There is very extensive metabolism of dibenzanthracene administered intraperitoneally, but it is by no means clear as yet what the exact mechanism of absorption and elimination by this route is. The B fractions of the urine and bile are unusually high, and there is a very appreciable I fraction in the feces. The very small amount of dibenzanthracene eliminated in the feces (1.2%) is in accord with the observations of Berenblum and Kendall (3a), who were unable to

detect any dibenzanthracene fluorescence in feces collected from mice that had been given the compound intraperitoneally.

Subcutaneous

Fractionations performed on the sites of subcutaneous injection after one week and three months, show that there is no appreciable degradation of the dibenzanthracene. It has already been demonstrated that most of the radioactivity lost from the site appears in the feces, and fractionation of the feces indicates a very substantial metabolism of the compound (only 11% of the radioactivity is due to the carcinogen). Thus it appears that the dibenzanthracene leaves the site of subcutaneous injection intact, but is then degraded during the process of elimination from the body.

Tumors induced by the labeled carcinogen

Several tumors, induced by the radioactive dibenzanthracene, have been fractionated to determine the extent of degradation. (For the percent of injected radioactivity in these tumors see Table VII (2).) There appears to be a considerable individual variation among these. In three of the tumors more than 90% of the radioactivity is due to unchanged dibenzanthracene. However, it is striking that in two of the tumors, the carcinogen has been extensively degraded (only 15% and 54% of the activity is due to dibenzanthracene). Since there is no evidence that under normal conditions any degradation occurs at the site of subcutaneous injection, these results suggest that neoplastic tissue is capable of degrading the carcinogen, whereas normal tissue cannot. Obviously, more experiments must be done before this point can be settled with certainty.

Photooxidation of dibenzanthracene

Labeled dibenzanthracene was photooxidized in benzene solution by exposure for one hour to a mercury vapor arc, according to the procedure of Alsopp and

Szigeti (7). The resulting mixture was processed in almost the usual way, except that Fraction B and C were not obtained, and Fraction C was a bicarbonate extraction. The products are unstable, for on acidification and warming of Fractions G and H as shown in Table VIII, radioactive carbon dioxide is eliminated. There is also an appreciable loss of overall activity when alkaline solutions are concentrated at atmospheric pressure, suggesting perhaps that volatile neutral substances are formed during the reaction. These observations suffice to prove that the water-soluble metabolites are not closely related to the photooxidation products, because the former never exhibit this instability.

Discussion of Results

In the preceding sections of this paper, evidence has been presented which proves that dibenzanthracene is metabolized by the mouse into at least four substances, and some speculations as to the sites of this degradation have been advanced. It is possible that a small amount of these compounds is produced as an artifcat from dihydroxydibenzanthracene during the isolation procedure.

The fact that unchanged dibenzanthracene is found in tumors six to eight months after their induction is an interesting one, and has previously been demonstrated spectrophotometrically by Lorenz and Shear (10). This observation, it must be emphasized, does not throw any light on the problem as to whether the original hydrocarbon is the true carcinogen, or whether some metabolite is. In view of the increasing body of evidence (11) that only very minute quantities of carcinogenic hydrocarbons are required to initiate the

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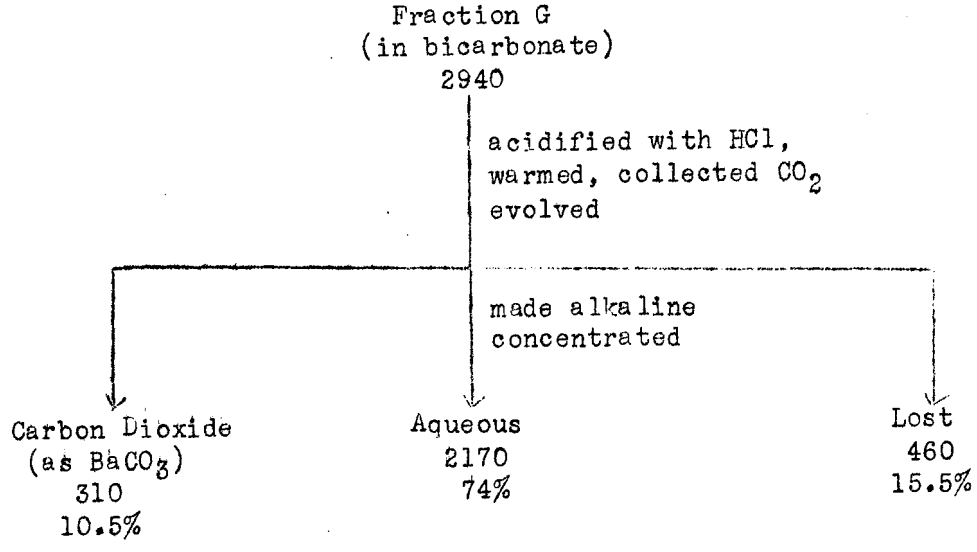
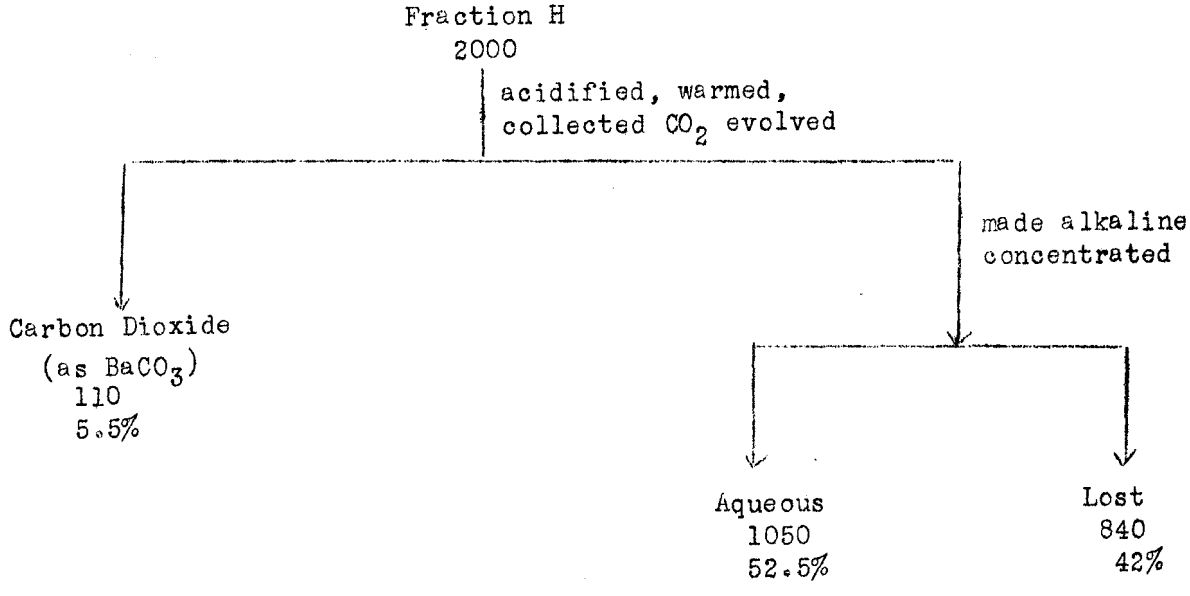
10. E. Lorenz and M. J. Shear, Am. J. Cancer 28, 333 (1936).

11. a) I. Berenblum, Arch. Path. 38, 233 (1944).
    b) W. F. Friedewald and P. Rous, J. Expt. Med. 80, 101 (1944).
    c) J. C. Mottram, Brit. J. Expt. Path 26, 1 (1945).
    d) I. Berenblum, Report at 4th International Cancer Congress, Sept. 1947.

TABLE VIII

PHOTOOXIDATION





irreversible process that leads to the tumor, the fact that appreciable amounts of dibenzanthracene are found in the tumor is of little more than academic interest. Indeed, it seems likely that the metabolic degradation described here is merely a detoxication process quite unrelated to the initiation of cancer.

This work is being continued with the aim of elucidation of the structures of these metabolites, and the various fractions described are being tested both for carcinogenic and tumor-regressive properties.