CV. STUDIES IN TISSUE METABOLISM.

II. THE INHIBITION OF LACTIC DEHYDROGENASE BY DERIVATIVES OF CARCINOGENIC COMPOUNDS.

By ERIC BOYLAND.

From the Research Institute, The Cancer Hospital (Free), London.

(Received May 1st, 1933.)

DURING the last few years, work on the chemistry of carcinogenesis has yielded pure hydrocarbons of considerable activity. The first pure hydrocarbon which was found to produce cancer was 1 : 2 : 5 : 6-dibenzanthracene [Cook *et al.*, 1932], and it was then shown that 5 : 6-cyclopenteno-1 : 2-benzanthracene [Cook, 1932] and some derivatives of 1 : 2 : 5 : 6-dibenzanthracene were carcinogenic when applied to the skin of mice. Cook, Hewett and Hieger [1933] have described the isolation, identification and synthesis of a very active carcinogenic compound present in coal tar, namely 1 : 2-benzpyrene. This has proved to be the most active carcinogenic substance yet known. Now that such active and well-defined substances which produce cancer are available it was thought that a study of the action of these on enzyme systems might give some indication of the biochemical changes occurring in tumour production.

Cancer is known to be produced not only by coal tar and the hydrocarbons mentioned but also by arsenic [Hutchinson, 1888]. Barry, Bunbury and Kennaway [1928] were able to show that arsenites inhibited xanthine oxidase, the oxidation of acetaldehyde by platinum and the oxidation of propaldehyde and acetaldehyde in the presence of glycine and phosphate. Szent-Györgyi [1930] also found that arsenites inhibited the respiration of liver and of yeast, addition of 0.00013 *M* arsenite causing 68 % decrease in the respiration of liver pulp. Banga and Szent-Györgyi [1932] have found that the part of the respiratory system of heart muscle which is inhibited by arsenite is concerned with lactate oxidation. They have succeeded in dividing this system into enzyme and coenzyme, and Banga, Szent-Györgyi and Vargha [1932] have described the preparation of this coenzyme. Some experiments recorded below show that arsenite inhibits lactic dehydrogenase, and that derivatives of carcinogenic hydrocarbons, which are chemically quite unrelated to arsenite, have a similar effect on this system. Thus derivatives of two distinct and chemically unrelated types of substance which produce cancer have the same action on this enzyme. The work of Warburg [1926] has shown that malignant tissue differs from most normal tissues in producing more lactic acid than it is able to oxidise, and hence is said to have a large aerobic glycolysis.

The work of Keilin [1929] has shown that the respiratory system of most cells can be divided into three parts: (1) indophenol oxidase, or "Atmungsferment" of Warburg, which activates oxygen; (2) cytochrome, a reversibly reducible pigment which acts as oxygen carrier; (3) the dehydrogenases, *e.g.* lactic dehydrogenase, which activates lactic acid so that it can reduce cytochrome or methylene blue. The respiration of tumours is greatly increased by

the addition of *p*-phenylenediamine, showing that there is ample indophenol oxidase or "respiratory enzyme" [Vietorisz, 1931; Boyland and Boyland, unpublished work]. Cytochrome has been found in most malignant tissues examined.

The defect of malignant tissue in lactate oxidation is therefore probably due to deficiency in dehydrogenases. Neither arsenite [Szent-Györgyi, 1930] nor the derivatives of carcinogenic hydrocarbons influence the action of indophenol oxidase, but they are able to inhibit the dehydrogenase systems in which cancer cells are deficient.

No claim is made here that the carcinogenic agents are the only substances which inhibit lactic dehydrogenase. Quastel and Wheatley [1931] found that some dyestuffs had a similar effect. Malachite green and acriflavine in dilutions of 1 in 5000 inhibited 90 % of the respiration of *B. coli* in the presence of lactate. Davies and Quastel [1932] showed that narcotics had a similar action. The results tabulated below show that some non-carcinogenic substances likewise inhibit lactic dehydrogenase.

EXPERIMENTAL.

Polycyclic hydrocarbons are insoluble in water. For experimental purposes solutions in benzene, toluene and *cyclohexane* were used. Measured amounts of such solutions were shaken with the enzyme preparation in phosphate buffer and one drop of capryl alcohol (to prevent frothing) in ordinary Thunberg tubes. The substrate and finally methylene blue solution were then added and the tube was evacuated. The reduction time at 37° was determined by observation in a glass-sided thermostat.

The preliminary experiments were carried out with 1 : 2 : 5 : 6-dibenzanthracene synthesised by the method described by Clar [1929]. This was dissolved in toluene to give a solution containing 0.1 g. per 100 cc. This was tested with (1) xanthine oxidase from milk, (2) lactic dehydrogenase of yeast, (3) glucose dehydrogenase from liver, (4) citric dehydrogenase from liver, and (5) succinic

Table I. *Effect of 1:2:5:6-dibenzanthracene on oxidising enzymes.*

Enzyme preparation	Substrate	Reduction time (in mins.) with		
		1 cc. 0.1 % 1 : 2 : 5 : 6-dibenzanthracene in toluene		
		1 cc. toluene	Not exposed to sunlight	After exposure to sunlight
Yeast lactic dehydrogenase (described in text, after Bernheim [1928])	Lactate <i>M</i> /80	10	10	16
Washed heart muscle, lactic dehydrogenase + coenzyme [Banga and Szent-Györgyi, 1932]	Lactate <i>M</i> /80	10	11	15½
Heart muscle, indophenol oxidase [Keilin, 1929]		See text		
Skeletal muscle, succinic dehydrogenase [Clutterbuck, 1928]	<i>M</i> /250 sodium succinate	9	9½	15
Liver, glucose dehydrogenase [Harrison, 1931]	<i>M</i> /40 glucose	10	10	11
Liver, citric dehydrogenase [Bernheim, 1928]	<i>M</i> /40 sodium citrate	10	10½	12
Milk, xanthine dehydrogenase [Dixon and Kodama, 1926]	<i>M</i> /8000 hypoxanthine	10	10½	12½

and lactic dehydrogenases of muscle. In each case the control tubes received a volume of toluene equal to that used in the experiment. No effect was obtained with freshly prepared solutions of the hydrocarbon when 2 cc. of enzyme in phosphate buffer, 1 cc. of substrate, 1 cc. of methylene blue (0.02 %), and 1 cc. of toluene solution were used. A solution of 1 : 2 : 5 : 6-dibenzanthracene which had stood in daylight for some days was found however to have a distinct inhibitory effect on lactic dehydrogenase and succinic dehydrogenase, but the other enzymes tested were less affected (Table I).

It is difficult to measure the activity of indophenol oxidase as the enzyme produces a blue colour in the presence of the cell debris, and only qualitative results were obtained. When the effect of irradiated 1 : 2 : 5 : 6-dibenzanthracene was tried on this enzyme derived from yeast cells it was found that the addition of the activated hydrocarbon appeared to increase the rate of production of blue colour from α -naphthol and *p*-phenylenediamine. This shows that the enzyme was not inhibited—the apparent increased activity was probably due to inhibition of dehydrogenases which normally reduce the amount of oxygen available for indophenol formation.

The "activation" of 1 : 2 : 5 : 6-dibenzanthracene was found to occur more rapidly in ultra-violet light. A solution which previously had no effect on lactic dehydrogenase had a marked inhibitory action after 2 hours' irradiation from a mercury arc lamp.

Preparation of lactic dehydrogenase.

For the subsequent experiments lactic dehydrogenase was used as here described. Bernheim [1928] prepared the enzyme from baker's yeast zymon by extraction with alkaline phosphate and dialysing to remove metabolites. The modified method which was used depended upon washing the zymon free from metabolites previous to extraction with phosphate. 10 g. baker's yeast zymon prepared according to details given by Harden [1932]¹ were washed 3 times by grinding with water and then centrifuging.

It was then found not to reduce methylene blue without the addition of lactate. The washed zymon was suspended in 100 cc. *M*/10 phosphate buffer p_H 8.4 and allowed to stand overnight between 0° and 5°. The suspension was then centrifuged, the clear liquid which contained lactic dehydrogenase was adjusted to p_H 7.4 and the reduction time of 2 cc. of the enzyme solution with 1 cc. *M*/20 lactate and 1 cc. 1 in 5000 methylene blue at 37.0° measured. The reduction time was generally under 10 minutes, and the enzyme was diluted carefully until the reduction time under these conditions was exactly 10 minutes. In this way a preparation with presumably a constant amount of enzyme is obtained.

In all experiments with inhibitors, the solutions were placed in Thunberg tubes in the following order:

- (1) 1 drop capryl alcohol;
- (2) inhibitor;
- (3) 2 cc. enzyme solution;
- (4) 1 cc. *M*/20 lithium lactate;
- (5) 1 cc. 1 in 5000 methylene blue.

If lactate is added before the inhibitor less inhibition is produced because the lactate protects the enzyme from inhibition. If methylene blue is added

¹ From baker's yeast kindly supplied by The Distillers' Co., Gt Burgh, Epsom.

before the lactate greater inhibition occurs, possibly because methylene blue itself destroys lactic dehydrogenase.

Solutions in benzene of a number of hydrocarbons¹ which have been tested for carcinogenic power were therefore irradiated and the irradiated solutions tested on lactic dehydrogenase. For irradiation, 10 cc. of a solution (0.01 % in benzene) of each hydrocarbon were placed in a transparent silica tube 10 inches from a vacuum-type vertical mercury vapour lamp². The tubes were left unstoppered open to the air. After 6 hours portions (0.2 cc.) of the solutions were added to 2 cc. of lactic dehydrogenase solution in *M*/15 phosphate buffer, p_H 7.4; lactate and methylene blue were then added, and the reduction time was observed (Table II). The method of indicating carcinogenic power by means

Table II. *Effect on lactic dehydrogenase of compounds irradiated for 6 hours in benzene solution.*

	Percentage inhibition	Results of test for carcinogenic activity
Benzene (control)	Nil	—
Anthracene	5	—
1:2-Benzanthracene	5	+
3:4-Benzphenanthrene	13	++
1:2:5:6-Dibenzanthracene	26	++++
5:6- <i>cyclo</i> Penteno-1:2-benzanthracene	24	++++
6:7- <i>cyclo</i> Penteno-1:2-benzanthracene	28	++
Pyrene	26	+
1:2-Benzpyrene	39	++++
4:5-Benzpyrene	20	— (at present)
Chrysene	17	+

of + signs is very rough, but it is extremely difficult to assess this action on a quantitative basis. The signs indicate the relative order of activity of the different compounds.

Although benzene is a convenient solvent from the point of view of stability and the ease with which the complex hydrocarbons dissolve in it, it has a very wide absorption band in the ultra-violet. For this reason, further experiments were carried out using *cyclohexane* as solvent. The same technique was employed with a larger range of substance (Table III). The tubes, each containing a different substance in solution, were irradiated in batches of ten at a time. With every batch irradiated in this way a tube containing anthracene was irradiated as control. In all the experiments there was good agreement between different determinations of the change with anthracene. Anthracene in *cyclohexane* was examined 10 times in this way and the percentage inhibitions produced after 24 hours' irradiation in successive experiments were 50, 42, 45, 54, 52, 48, 43, 40, 46 and 41. The effect of the photochemically oxidised substances on the enzyme was always determined in duplicate and repeated if agreement within 5 % was not obtained. The first part of Table III contains data referring to hydrocarbons which have been tested for carcinogenic activity and found to give negative results. (Some of the mouse-painting tests of compounds occurring in the first part of Table III are not yet complete. The designation of the substances as non-carcinogenic is subject to the possible future

¹ I am indebted to Dr J. W. Cook for specimens of over 50 of the hydrocarbons used in this work. The syntheses of 33 of these are described in a series of papers by him in the *Journal of the Chemical Society*, 1931 and 1932.

² As supplied by the Thermal Syndicate.

Table III. *Percentage inhibition of lactic dehydrogenase produced by 0.2 cc. 0.01 % cyclohexane solutions of various hydrocarbons irradiated in ultra-violet light.*

	Hours' irradiation			
	0	3	6	24
<i>Non-carcinogenic hydrocarbons:</i>				
Naphthalene	0	7	9	14
Acenaphthene	2	6	—	19
Anthracene	0	10	22	46
Phenanthrene	0	10	12	20
Triphenylene	2	7	11	25
Naphthacene	0	3	15	26
Fluoranthene	0	5	8	10
Retene	0	0	7	10
3'-Methyl-1:2-benzanthracene	0	0	6	14
7-Methyl-1:2-benzanthracene	0	0	4	11
2'-Methyl-1:2-benzanthracene	2	0	12	10
3-Methyl-1:2-benzanthracene	0	3	3	5
2':7-Dimethyl-1:2-benzanthracene	0	5	2	10
3':6-Dimethyl-1:2-benzanthracene	2	3	8	15
3':7-Dimethyl-1:2-benzanthracene	0	14	8	13
2':6-Dimethyl-1:2-benzanthracene	2	4	8	11
3-isoPropyl-1:2-benzanthracene	0	0	0	0
7-isoPropyl-1:2-benzanthracene	2	5	7	17
10-isoPropyl-1:2-benzanthracene	0	4	0	28
6-Phenyl-1:2-benzanthracene	0	4	10	8
10-Benzyl-1:2-benzanthracene	0	5	0	12
9:10-Diphenyl-1:2-benzanthracene	0	2	5	5
Perylene	0	8	15	21
1:2:3:4-Dibenzanthracene	0	0	17	20
1:2:6:7-Dibenzanthracene	0	5	5	16
3:4:5:6-Dibenzphenanthrene	0	7	9	18
1':2'-Naphtha-1:2-anthracene	0	10	6	20
2':3'-Naphtha-1:2-phenanthrene	2	8	22	24
2':3'-Naphtha-1:2-anthracene	0	7	16	25
4:5-Benzpyrene	0	6	11	25
7:7'-Dimethyl-2':3'-naphtha-1:2-anthracene	0	7	12	23
1:2:3:4:5:6-Tribenzanthracene	0	4	10	16
2':1'-Anthra-1:2-anthracene	0	11	15	26
1':2'-Anthra-1:2-anthracene	2	8	19	22
1:2:1':2'-Dibenz-6:6' (or 7:7')-dianthryl	0	5	5	10
Dinaphthachrysene	0	5	8	30
<i>Substances which have produced papillomata only:</i>				
Pyrene	0	16	7	14
Chrysene	0	4	5	9
1:2:7:8-Dibenzanthracene	2	7	9	11
<i>Carcinogenic substances:</i>				
1:2-Benzanthracene	0	5	—	21
6-Methyl-1:2-benzanthracene	2	6	—	19
6:7-Dimethyl-1:2-benzanthracene	0	12	—	16
6-isoPropyl-1:2-benzanthracene	0	4	—	35
1:2:5:6-Dibenzanthracene	0	4	—	36
2'-Methyl-1:2:5:6-dibenzanthracene	0	10	—	48
3'-Methyl-1:2:5:6-dibenzanthracene	0	2	—	28
9-Amino-1:2:5:6-dibenzanthracene	0	0	—	41
9-Methoxy-1:2:5:6-dibenzanthracene	0	6	—	40
9:10-Dibenzyl-1:2:5:6-dibenzanthracene	0	4	—	35
5:6-cycloPenteno-1:2-benzanthracene	0	6	—	35
Phenanthra-acenaphthene	2	6	—	34
1:2-Benzpyrene	0	8	—	37
3:4-Benzphenanthrene	0	2	—	34
<i>Biological products:</i>				
Ketohydroxyoestrin	0	—	—	36
Cholesterol	0	—	—	32
Ergosterol	0	—	—	20
Oleic acid	0	—	—	34
Glucose	0	—	—	Nil
Hypoxanthine	0	—	—	11
Glutathione	0	—	—	11

production of tumours in the mice under test.) Of these, anthracene produces the greatest inhibitory action. Anthracene irradiated in benzene does not have so large an effect; it is known to polymerise to dianthracene to a greater extent in benzene than in other solvents. The table shows that apart from anthracene 4 only of the 47 non-carcinogenic hydrocarbons in the amounts used (0.2 cc. of 1 in 10,000 solution irradiated for 24 hours) produce more than 25 % inhibition, the maximum figure being 30 % (dinaphthachrysene).

On the other hand, turning to the second part of Table III which refers to known carcinogenic compounds it is seen that most of the carcinogenic substances produce over 30 % inhibition under the same conditions. Thus it can be said that on the whole carcinogenic compounds are more readily changed to give substances which have the power of inhibiting lactic dehydrogenase, but there is no close parallelism. Thus one of the least active cancer-producing compounds (2'-methyl-1:2:5:6-dibenzanthracene, 48 % inhibition) yields more of the inhibitory product than does one of the most active (1:2-benzpyrene, 37 % inhibition).

The concluding section of Table III contains substances of biological interest which were examined in the same way. I am indebted to Dr G. F. Marrian for the sample of ketohydroxyoestrin used in the experiment. It will be noticed that after irradiation both cholesterol and ketohydroxyoestrin produce inhibition of lactic dehydrogenase to an extent comparable with that produced by the carcinogenic substances. It is, however, certain that cholesterol, which is widely distributed in the tissues of normal animals, is not carcinogenic.

The effect of different solvents.

The case of anthracene shows that the reaction which the hydrocarbons undergo is not independent of the solvent used, as anthracene is much more readily changed in *cyclohexane* than in benzene. In order to study the effect of different solvents, 1:2:5:6-dibenzanthracene was irradiated in the solvents shown in Table IV. The amounts of the various reagents were those stated on

Table IV. *Effect on lactic dehydrogenase of 1:2:5:6-dibenzanthracene irradiated with ultra-violet light for 24 hours in the presence of air.*

1 mg. 1:2:5:6-dibenzanthracene irradiated in 10 cc. solvent.

Solvent	Percentage inhibition by 0.2 cc. solution	Absorption band of solvent between 2000 Å. and 4000 Å. (data from literature)
Methyl alcohol	16	Very slight general absorption
Ethyl alcohol	35	Very slight general absorption
Acetone	37	2300-2950
Methylethylketone	64	2320-2980
Hexane	22	Very slight
<i>cyclo</i> Hexane	31	Slight
Benzene	40	2300-2750
Toluene	42	2200-2650
<i>o</i> -Xylene	41	2600-2800
<i>m</i> -Xylene	24	2600-2800
<i>p</i> -Xylene	58	2600-2800 + large band at 2800
Nitrobenzene	13	Very slight
Amyl acetate	56	Large band below 4000
Carbon tetrachloride	28	Weak general absorption

p. 793. The most effective solvents are apparently methylethylketone, *p*-xylene and amyl acetate. It is remarkable that there should be such large differences between *o*-, *m*- and *p*-xylenes.

The more effective solvents for the reaction have very marked absorption bands in the ultra-violet. Hexane, methyl alcohol and nitrobenzene, which are very transparent to ultra-violet light between 2000 Å. and 3000 Å., give very small amounts of "activated" hydrocarbon; in this respect ethyl alcohol is an exception. The difference in absorption spectra between *p*-xylene on the one hand and *o*- and *m*-xylene on the other is considerable [Baly and Tryhorn, 1915], in that *p*-xylene has a very strong band at 2800 Å., while the *o*- and *m*- isomerides have no such bands. Similarly methylethylketone absorbs more between 2200 Å. and 3000 Å. than does acetone [Bielecki and Henri, 1912]. The absorption bands of polycyclic hydrocarbons also occur between 2000 Å. and 3000 Å., so that it might be expected that the solvents which absorb ultra-violet light of that wave length would hinder the reaction, but this is not so.

Regener [1906] showed that ozone was decomposed by light of 2000–3000 Å. and Schumann [1903] found that oxygen was converted into ozone by light of shorter wave-length than 1950 Å. Experiments described below show that oxygen is necessary for the "activation" of hydrocarbons, and it is possible that the rôle of ultra-violet light is to convert O_2 into O_3 , which might combine with the hydrocarbons to form an ozonide or peroxide. Such an explanation would be in agreement with the results obtained on comparing the effect of different solvents.

Nature of the active agent.

The preparation of aqueous colloidal suspensions of 1 : 2 : 5 : 6-dibenzanthracene has been described elsewhere [Boyland, 1932]. Such solutions were found to be completely inactive towards lactic dehydrogenase even after long exposure to daylight. Under such conditions the hydrocarbon could come into perfect physical contact with the enzyme, and hence the result shows that on irradiation some chemical change must occur. After irradiation in benzene or cyclohexane the solutions of carcinogenic hydrocarbons lose fluorescence and become yellow or slightly brown.

Solutions of 1 : 2 : 5 : 6-dibenzanthracene and 1 : 2-benzpyrene in benzene were boiled to remove dissolved oxygen and then irradiated in closed quartz tubes in the presence of nitrogen for 24 hours. The solutions were then colourless and strongly fluorescent, while control solutions which had been irradiated while exposed to the air were yellow and less fluorescent. The solutions irradiated anaerobically were free from inhibitory action towards lactic dehydrogenase, while those irradiated in contact with air were actively inhibitory. This indicates that the active inhibitory substances are oxidation products.

The fact that these substances inhibit water-soluble enzymes suggests that the product is water-soluble. To test this, a benzene solution of 1 : 2 : 5 : 6-dibenzanthracene which had been oxidised in air in ultra-violet light was extracted with aqueous solutions of different p_H , the extracts were neutralised and tested with lactic dehydrogenase. The results are given in Table V and Fig. 1. The active extracts made with alkaline solutions were brown in colour and the depth of colour appeared to follow the degree of activity.

Carcinogenic hydrocarbons are therefore capable of oxidation in ultra-violet light to acidic substances, which can inhibit dehydrogenating enzymes. Although the substances react as acids in their solubility relationships it is not certain that they are carboxylic acids. Qualitative experiments showed that the oxidised product could liberate iodine from hydriodic acid in glacial acetic acid solution.

Table V. *Effect of p_H on the amount of inhibitor extracted from a photochemically oxidised solution of 1:2:5:6-dibenzanthracene in benzene.*

Solution used for extraction	p_H of solution	Percentage inhibition produced by 0.5 cc.
Water	6	10
N/10 acetic acid	3	10
M/10 KH_2PO_4	5	24
M/10 phosphate p_H 7.0	7	28
M/10 Na_2HPO_4	8	35
M/10 K_2CO_3	10	49
N/100 NaOH	12	58

Fig. 1 (see Table V).

Hartmann and Siebert [1932] and Hock and Sosemihl [1933] have described the isolation of tetrahydronaphthalene peroxide produced by oxidation of tetrahydronaphthalene with air, and this compound forms a water-soluble sodium salt, while the free peroxide is insoluble in water. It is conceivable that the oxidation of carcinogenic hydrocarbons yields a peroxide and that it is this compound which is able to destroy dehydrogenating enzymes. So far all attempts to isolate the pure inhibitory compound produced by oxidation of 1:2:5:6-dibenzanthracene have been unsuccessful, although qualitative evidence for the production of 1:2:5:6-dibenz-9:10-anthraquinone and a dicarboxylic acid has been obtained. That the inhibiting substance is not a dicarboxylic acid produced by opening of one of the rings is made probable by the fact that phthalic acid and naphthalene-1:2-dicarboxylic acid have no inhibitory power, and more complex dicarboxylic acids such as deoxybilianic acid and isodeoxybilianic acid show but slight activity. The active product is not so stable in aqueous solution as would be expected of a dicarboxylic acid.

As it was known that the active inhibitory principle produced from the hydrocarbons was alkali-soluble, solutions of 1:2:5:6-dibenzanthracene in benzene and toluene were exposed to ultra-violet light while being incubated over dilute sodium carbonate solution. The solutions were irradiated in quartz tubes and in some experiments the lower aqueous layer was shaded from the ultra-violet light, so that the alkali-soluble product would be less liable to change. In all experiments of this nature in which water or alkaline solutions were left in contact with the hydrocarbon solution, much smaller yields of inhibiting substance were obtained. The presence of water appeared to interfere with the oxidation process. This again suggests that the action is due to ozone, for it is well known that water tends to decompose ozone.

Effect of ozone on hydrocarbons.

Ozone was made by passing oxygen through a tube subjected to a silent electrical discharge. The ozonised oxygen was passed through the liquid under treatment at a constant rate. Solutions treated with ozone in this way were then tested for inhibitory action on lactic dehydrogenase. Controls were carried out by treating the solvent used with ozone for the same time as the solution. *cyclo*Hexane was found to be apparently unaffected by ozone when treated in this way, but solutions of 1 : 2-benzpyrene in benzene or *cyclo*hexane quickly changed, becoming yellow-coloured and practically non-fluorescent. Similar solutions of 1 : 2 : 5 : 6-dibenzanthracene did not change so rapidly, but even in this case after passing ozone for 3 hours the dibenzanthracene became oxidised and then caused inhibition of lactic dehydrogenase to the extent shown in Table VI.

Table VI. *Effect on lactic dehydrogenase of 1:2-benzpyrene and 1:2:5:6-dibenzanthracene in benzene oxidised with ozone.*

	Reduction time mins.	Percentage inhibition
0.4 cc. benzene	10	—
0.1 cc. 0.1 % 1:2-benzpyrene	10½	5
0.2 "	15	33
0.4 "	16½	40
0.8 "	22	55
0.1 cc. 0.1 % 1:2:5:6-dibenzanthracene	10	Nil
0.2 "	11½	13
0.4 "	12	17
0.8 "	15½	36

There is no doubt that ozone is able to oxidise these two carcinogenic hydrocarbons. Possibly an ozonide resembling the anthracene ozonide of Molinari [1907] is formed, but this may in turn change to some other oxidation product.

Comparison of effect with that of arsenite.

The inhibitory effect of arsenite on oxidations has been known since the work of Barry, Bunbury and Kennaway [1928]. The effects of arsenite and oxidised 1 : 2 : 5 : 6-dibenzanthracene were therefore compared quantitatively. The same standard yeast lactic dehydrogenase was used with the concentrations of sodium arsenite shown in Table VII.

Table VII. *Effect of sodium arsenite on the action of dehydrogenase in presence of M/80 lactate.*

Concentration of NaAsO ₂	Reduction time mins.	Percentage inhibition
Nil	10	—
M/660	10½	5
M/330	13	23
M/180	15½	36
M/90	20	50

Addition of M/90 arsenite thus appears to inactivate half the enzyme when the lactate is present in the concentration shown. Table IV shows that 0.2 cc. of 0.01 % 1 : 2 : 5 : 6-dibenzanthracene irradiated for 24 hours in methylethylketone, *p*-xylene or amyl acetate causes more than 50 % inhibition. This is a final concentration equivalent to 0.005 % of the original hydrocarbon or

0.00018 *M*. Thus a concentration approximately only one-sixtieth of that necessary with arsenite inactivates one-half of the enzyme. Activated 1 : 2 : 5 : 6-dibenzanthracene is therefore 60 times as active an inhibitor as arsenite when compared in presence of *M*/80 lactate.

Arsenite is a competitive inhibitor [*cf.* Haldane, 1930] as is shown by the results in Table VIII.

Table VIII. *Effect of lactate concentration on inhibition by arsenite.*

Concentration of lactate	Reduction time (mins.)		Percentage inhibition by arsenite
	Without arsenite	With <i>M</i> /60 NaAsO ₂	
<i>M</i> /10	9	18½	52
<i>M</i> /20	9½	25	68
<i>M</i> /40	10½	45	79
<i>M</i> /80	12	80	84
<i>M</i> /160	15	120	97

Results of a similar experiment using oxidised 1 : 2 : 5 : 6-dibenzanthracene are shown below (Table IX).

Table IX. *Effect of lactate concentration on inhibition by oxidised 1 : 2 : 5 : 6-dibenzanthracene.*

Concentration of lactate	Reduction time without oxidised 1 : 2 : 5 : 6-dibenzanthracene mins.	Reduction time with 0.2 cc. 0.01 % oxidised 1 : 2 : 5 : 6-dibenzanthracene in benzene mins.	Percentage inhibition
<i>M</i> /4	6½	14½	53
<i>M</i> /8	6	11	48
<i>M</i> /16	5	11	55
<i>M</i> /25	5½	13½	59
<i>M</i> /50	6	15	59
<i>M</i> /100	6½	17½	62
<i>M</i> /200	8	35	78

The inhibition does not vary much with change of substrate concentration and would therefore appear to be of a non-competitive type. The oxidised hydrocarbon destroys or inactivates a definite amount of enzyme and therefore differs in its effect from that of arsenite which competes with the substrate.

DISCUSSION.

The results in Tables I and II seemed to show that carcinogenic hydrocarbons could be photochemically oxidised to products which destroy dehydrogenating enzymes. In these preliminary experiments the effect seemed to run parallel with the carcinogenic activity of the substance when the oxidation was carried out with only a few substances in benzene solution. It might therefore have been a useful quick test of carcinogenic power, but further work on over 50 substances in *cyclohexane* showed the parallelism to be very rough, as anthracene and cholesterol, which are non-carcinogenic, were oxidised to powerful inhibitory products.

It is possible that this effect on dehydrogenases might explain the mechanism of cancer production by carcinogenic hydrocarbons. If the dehydrogenating enzymes in the tissues were destroyed there would be a tendency to local accumulation of lactic acid. Tumour cells survive and grow in media containing

lactic acid and are probably less affected by it than normal cells. If in tissue in which dehydrogenases were destroyed a malignant cell were to appear it would be at a great advantage and would grow at the expense of normal cells. It is possible that the active oxidation product of the hydrocarbons studied is of a peroxide nature which destroys the enzyme by oxidation. Such an oxidation to a peroxide may occur in the body and the oxidative destruction of the enzyme by this might yield a product capable of reoxidation to an active product. By this means a small quantity of hydrocarbon may inactivate many molecules of enzyme in the body. The possible biological oxidation of carcinogenic hydrocarbons will be studied in the near future.

SUMMARY.

Many polycyclic hydrocarbons are oxidised photochemically in air to products which are able to destroy dehydrogenating enzymes. On the whole, carcinogenic hydrocarbons, such as 1 : 2 : 5 : 6-dibenzanthracene and 1 : 2-benzpyrene, after oxidation are capable of greater inhibition of lactic dehydrogenase than are the non-carcinogenic hydrocarbons.

REFERENCES.

- Baly and Tryhorn (1915). *J. Chem. Soc.* **107**, 1058.
 Banga and Szent-Györgyi (1932). *Biochem. Z.* **246**, 203.
 ——— and Vargha (1932). *Z. physiol. Chem.* **210**, 227.
 Barry, Bunbury and Kennaway (1928). *Biochem. J.* **22**, 1102.
 Bernheim (1928). *Biochem. J.* **22**, 1178.
 Bielecki and Henri (1912). *Compt. Rend. Acad. Sci.* **155**, 456.
 Boyland (1932). *Lancet*, ii, 1108.
 Clar (1929). *Ber. deutsch. chem. Ges.* **62**, 350.
 Clutterbuck (1928). *Biochem. J.* **22**, 1193.
 Cook (1932). *Proc. Roy. Soc. Lond.* B **111**, 489.
 ——— Hewett and Hieger (1933). *J. Chem. Soc.* 395.
 ——— Hieger, Kennaway and Mayneord (1932). *Proc. Roy. Soc. Lond.* B **111**, 455.
 Davies and Quastel (1932). *Biochem. J.* **26**, 1672.
 Dixon and Kodama (1926). *Biochem. J.* **20**, 1104.
 Haldane (1930). *Enzymes*. (Longmans, Green and Co., London.)
 Harden (1932). *Alcoholic fermentation*. (Longmans, Green and Co., London.)
 Harrison (1931). *Biochem. J.* **25**, 1016.
 Hartmann and Siebert (1932). *Helv. Chim. Acta*, **15**, 1390.
 Hock and Sosemihl (1933). *Ber. deutsch. chem. Ges.* **66**, 61.
 Hutchinson (1888). *Trans. Path. Soc. Lond.* **39**, 352.
 Keilin (1929). *Proc. Roy. Soc. Lond.* B **104**, 206.
 Molinari (1907). *Ber. deutsch. chem. Ges.* **40**, 4154.
 Quastel and Wheatley (1931). *Biochem. J.* **25**, 629.
 Regener (1906). *Ann. Physik.* **20**, 1033.
 Schumann (1903). *Smithsonian Contrib. Knowledge*, 29.
 Szent-Györgyi (1930). *Biochem. J.* **24**, 1723.
 Vietoris (1931). *Biochem. Z.* **240**, 488.
 Warburg (1926). *Stoffwechsel der Tumoren*. (Springer, Berlin.)