XXXIII. STUDIES IN TISSUE METABOLISM.

III. THE EFFECT OF OXIDISED

1:2:5:6-DIBENZANTHRACENE.

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By means of the Thunberg technique oxidation products of carcinogenic hydrocarbons were shown to inhibit lactic and succinic dehydrogenases of yeast and muscle extracts in the presence of methylene blue as hydrogen acceptor [Boyland, 1933]. Qualitative examination of the effect on the oxidation of *p*-phenylenediamine by washed tissue indicated that these hydrocarbons did not inhibit indophenol oxidase. The enzymes used in the previous experiments had been used in the form of extracts. The object of the present work was the investigation of the effect of a water-soluble oxidation product of the carcinogenic hydrocarbon, 1:2:5:6-dibenzanthracene [Cook *et al.*, 1932] on the respiration and anaerobic glycolysis of surviving animal tissues (normal organs and tumours of rat and mouse) using the Warburg technique. The tissues were suspended in Ringer-phosphate or *M*/15 phosphate solutions (p_H 7.3), either in slices less than 0.4 mm. thick or finely minced, in the vessels of a Warburg apparatus; the amounts of oxygen absorbed and the anaerobic glycolysis were measured in the presence of varying concentrations of the oxidation product.

EXPERIMENTAL.

1:2:5:6-Dibenzanthracene (0.1 % in benzene) was oxidised by passing a stream of oxygen through the solution while exposed to ultra-violet light for 48 hours. A light brown precipitate was formed, and the solution became darker. The precipitate and solution were extracted with aqueous 2*N* NaOH, and the extract was precipitated with cold hydrochloric acid. The brown precipitate was washed with water and dried; weighed quantities were dissolved either (1) in phosphate buffer solution (*M*/5; final p_H 7.3) for determination of respiration or (2) in 0.82 % Na_2CO_3 solution, neutralised with CO_2 gas, for measurement of glycolysis. In some cases the precipitate never entirely dissolved; this was due to the instability of the oxidation product, which in certain circumstances partially changes to an insoluble substance. Concentrations quoted below are therefore only approximate.

Measurement of metabolism.

The standard Warburg apparatus was used, with simple conical vessels with inset trough and side-arm. For respiration, 0.1 ml. of 2*N* NaOH was placed in the central trough, and the tissue in *M*/15 phosphate or Ringer-phosphate was contained in the main vessel. In some cases the oxidised dibenzanthracene solutions and substrate were also placed in the main vessel. When tissue slices were used it was however found advisable to measure the respiration in Ringer

alone for a certain time ($\frac{1}{2}$ to 1 hour) before adding the substrates and different concentrations of oxidation product from the side vessels. The reason for this was that variations up to 15 % occurred in the respiration of tissues from any one animal. Brain, and tumours containing much blood or necrotic material, showed variations outside these limits. The respiration was in all cases measured in oxygen which was passed through the vessels for 20 seconds before closing the taps. Tissues were minced finely with scissors and 0.1 or 0.2 g. suspended in $M/15$ phosphate in the Warburg vessels, or slices were prepared according to Warburg [1923] and the dry weights determined at the end of the experiment. In each set of experiments there was one thermobarometer, one vessel containing a sample of tissue in Ringer-phosphate (or phosphate) alone and another the same with lithium lactate or sodium succinate (final concentration $M/100$). The remaining vessels contained in addition varying concentrations of oxidised dibenzanthracene.

A typical experiment was as follows:

Vessel No.	Main vessel Ringer-phosphate ml.	Side vessel			Inset 2N NaOH ml.
		Lactate ml.	Oxidised dibenzanthra- cene in phosphate ml.	Phosphate ml.	
1	1	—	—	—	—
2	0.6	—	—	0.3	0.1
				+ 0.1 H ₂ O	
3	0.6	0.1	—	0.3	0.1
4	0.6	0.1	0.1	0.2	0.1
5	0.6	0.1	0.2	0.1	0.1
6	0.6	0.1	0.3	—	0.1

The vessels after being filled with oxygen were shaken for 15 minutes in the thermostat to reach temperature equilibrium, and then readings were taken every 10 or 15 minutes, generally for an hour, before tipping in the solutions from the side vessels. When minced tissue was used it was found unnecessary to determine the normal respiration as there were negligible differences between duplicate experiments; in these cases all the solutions were from the beginning in the main vessels with the tissue. For determination of glycolysis, the tissue was suspended in Ringer-bicarbonate containing glucose, and the dibenzanthracene derivative was dissolved in bicarbonate. A mixture of 95 % nitrogen and 5 % carbon dioxide was passed through the vessels for a minute at the beginning of an experiment.

A few sets of experiments on respiration and glycolysis were carried out under aseptic conditions, and cultures were made at the end of the experiment to test the sterility. In no case did the comparison of these and other experiments show differences which could be ascribed to bacterial contamination.

RESULTS.

A. Normal tissues.

Muscle. Minced voluntary muscle of the rat in $M/15$ phosphate was used for the determination of respiration and anaerobic glycolysis at 25°. In one series of experiments on respiration, diaphragm muscle (rat) at 37.5° in Ringer-phosphate was used.

The tables show that a concentration of 0.02 % of oxidised dibenzanthracene reduced the oxygen consumption by a third in the first hour, and 0.3 % by 55 %.

Table I. *Minced rat muscle in M/15 phosphate (p_H 7.3) at 25°.*

Hour	Q_{O_2} Concentration of oxidised dibenzanthracene			
	0	0.01 %	0.02 %	0.08 %
1	37.5	31.0	25.0	20.2
2	18.7	17.5	11.2	7.2
3	7.0	7.5	4.0	4.0

Table II. *Minced rat muscle in Ringer at 25°.*

Hour	Q_{O_2} in Ringer-phosphate Concentration of oxidised dibenzanthracene		
	0	0.1 %	0.3 %
1	25.0	14.3	11.3
2	20.5	9.5	4.0
3	12.8	7.5	2.0

Hour	$Q_{M}^{N_2}$ in Ringer-bicarbonate Concentration of oxidised dibenzanthracene			
	0	0.1 %	0.2 %	0.3 %
1	21.3	18.8	20.0	4.6
2	7.5	6.3	3.8	0.0

Table III. *Rat diaphragm in Ringer-phosphate at 37.5°.*

Hour	Q_{O_2}			
	11.6	11.5	12.5	10.0
	Concentration of oxidised dibenzanthracene			
	0	0.03 %	0.06 %	0.09 %
2	9.7	9.0	7.7	5.7
3	8.5	8.6	5.8	5.2
4	7.9	7.5	5.4	3.8
	% decrease calculated on 1st hour			
2	16	22	38	43
3	27	25	54	48
4	33	35	57	62

In 3 hours this concentration almost stopped respiration and had completely arrested glycolysis within 2 hours.

As the glycolysis of minced rat muscle was inhibited by the oxidised 1:2:5:6-dibenzanthracene, a few experiments on the effect of the substance on glycolysis of rat muscle extract were carried out, with the co-operation of Dr C. A. Mawson. The muscle extract was made according to the method used by Meyerhof [1930]; although Meyerhof states that rat muscle yields an inactive extract Mawson (unpublished results) has been able to obtain glycolytically active rat muscle extracts. The results of experiments on undialysed and dialysed extracts showing pronounced inhibition of the lactic acid formation are given in Tables IV (a) and (b).

Table IV (a). 5 ml. undialysed rat muscle extract incubated 2 hours at 37°.

	Oxidised 1:2:5:6- dibenz- anthracene %	mg. lactic acid	% inhibi- tion of lactic acid formation
Unincubated		9.76	
Incubated with adenylypyrophosphate + MgCl ₂		10.13	
" " + glycogen		17.64	
" " "	+0.002	17.37	3.6
" " "	+0.004	16.05	21
" " "	+0.012	11.85	77

Table IV (b). 5 ml. rat muscle extract dialysed for 3 hours at 0°, incubated 2 hours at 37°.

	Oxidised 1:2:5:6- dibenz- anthracene %	mg. lactic acid	% inhibi- tion of lactic acid formation
Unincubated extract		1.34	
Incubated with adenylypyrophosphate and MgCl ₂		1.66	
" " + glycogen		5.12	
" " "	+0.002	4.39	21
" " "	+0.004	3.27	54
" " "	+0.012	2.37	78

Liver. All samples in the following tables, except those marked "No lactate" (or "No succinate") had an addition of *M*/100 lactate (or *M*/100 succinate) after an hour.

Table V. Rat liver slices in Ringer-phosphate at 37°.

Hour	Q_{O_2}				
	Concentration of oxidised dibenzanthracene				
	No lactate 0	0	0.002 %	0.005 %	0.01 %
1	4.3	7.3	7.5	6.2	3.4
2	3.4	6.6	6.5	5.0	2.0
3	3.3	6.2	5.0	4.7	1.6

Table VI. Rat liver slices in Ringer-phosphate at 37°.

Hour	Q_{O_2}				
	Concentration of oxidised dibenzanthracene				
	No lactate 0	0	0.1 %	0.2 %	0.3 %
1	9.3	9.1	8.2	9.3	8.3
2	9.3	10.0	8.6	9.8	7.1
3	8.5	8.7	8.1	8.9	6.2
4	7.8	8.1	7.5	7.6	5.7
% difference, calculated on 1st hour					
2	0	+10	+5	+5	-14
3	-8	-4	-1	-4	-25
4	-16	-11	-8	-18	-31

Tables V and VI are typical of ten experiments on liver. Table V shows that the respiration was increased 70 % by the addition of lactate, and that the dibenzanthracene derivative greatly reduced the oxygen uptake. In the experiment represented by Table VI, on the other hand, the respiration was very slightly increased by lactate, and the inhibition was also less. The mean of six sets of experiments shows almost equal increase of respiration due to added lactate, and decrease due to the presence of oxidised dibenzanthracene, indicating that it is mainly lactic oxidation which is inhibited.

Kidney. Sections from rat kidney cortex were used to investigate the effect of the oxidised hydrocarbon on lactate and on succinate oxidation. As the normal respiration of kidney is higher than that of liver thinner sections (0.15–0.3 mm.) were used.

Fig. 1. Respiration of kidney slices in Ringer-phosphate at 37.5°.

Table VII. *Rat kidney slices in Ringer-phosphate (with lactate) at 37.5°.*

Hour	Q_{O_2}									
	20.7	19.0	21.5	22.5	20.0	22.5	22.6	25.5	21.9	
	Concentration of oxidised dibenzanthracene									
	No lactate	0	0	0.005 %	0.01 %	0.02 %	0.05 %	0.1 %	0.2 %	
1	16.2	26.5	25.5	28.0	23.5	23.0	22.2	25.0	17.8	
2	8.3	17.2	20.0	20.2	16.5	16.8	15.0	17.5	12.2	
	% difference calculated on 1st hour									
2	-22	+40	+19	+22	+17	+2	-2	-2	-19	
3	-60	-9	-7	-11	-17	-25	-34	-31	-45	

Table VIII. *Rat kidney slices in Ringer-phosphate (with succinate) at 37.5°.*

Hour	Q_{O_2}				
	16.8	18.0	17.8	15.5	16.8
	Concentration of oxidised dibenzanthracene				
	No succinate	0	0.017 %	0.033 %	0.05 %
2	13.9	31.5	25.1	18.8	21.8
3	8.8	16.5	15.0	11.2	11.8
	% difference, calculated on 1st hour				
2	-17	+75	+41	+21	+30
3	-48	-8	-16	-28	-30

Tables VII and VIII show that a concentration of 0.02 % of the benzanthracene derivative has a measurable effect on lactate oxidation. Table VII shows that within the experimental error increasing concentrations from 0.001 up to 0.2 % show a steadily increasing effect. A concentration of 0.033 % has an inhibitory effect on succinic oxidation.

Brain. A modification of the technique used by Quastel [1932] was employed in a few sets of experiments on minced rat's brain. The tissue was allowed to respire in phosphate solution for 2 hours before the lactate and the solutions of oxidised dibenzanthracene were added. The respiration fell rapidly during the first hour and then became more uniform, though still falling slowly. The normal respira-

 Table IX. *Rat brain minced in M/15 phosphate (dry weights taken as 1/5 wet weights) at 37.5°.*

Hour	Q_{O_2}				
	19.2	17.7	17.0	18.0	17.5
	Concentration of oxidised dibenzanthracene				
	No lactate	0	0.005 %	0.01 %	0.02 %
3	11.1	18.5	21.2	17.9	16.5
4	8.1	16.8	19.8	17.2	11.8
	% difference, calculated on 2nd hour				
3	-42	+4	+25	-1	-5
4	-58	-5	+16	-4	-33

 Table X. *Rat brain slices in Ringer-phosphate (from dry weights, brain dried at 50°) at 37.5°.*

Hour	Q_{O_2}				
	6.0	5.0	6.5	7.8	5.1
	Concentration of oxidised dibenzanthracene				
	No lactate	0	0.017 %	0.033 %	0.05 %
2	3.3	5.4	6.3	8.0	4.2
3	1.4	4.3	5.0	5.8	2.6
	% difference, calculated on 1st hour				
2	-45	+8	-3	+2	-18
3	-77	-14	-23	-25	-49

Table XI. *Rat brain slices in Ringer-bicarbonate (from dry weights, brain dried at room temperature) at 37.5°.*

Hour	$Q_M^{N_2}$ Concentration of oxidised dibenzanthracene			
	0	0.03 %	0.1 %	0.3 %
1	5.7	6.4	4.4	3.9
2	3.6	3.9	2.3	1.5

Table XII. *Rat brain slices in Ringer-bicarbonate (from dry weights, brain dried at room temperature) at 37.5°.*

Hour	$Q_M^{N_2}$ Concentration of oxidised dibenzanthracene			
	0	0.03 %	0.1 %	0.3 %
1	6.6	6.0	6.2	5.4
2	5.6	4.3	5.0	3.5

tion was taken as that during the second hour and the respiration in subsequent hours compared with it. Respiration and glycolysis were also investigated for brain slices in Ringer. As the glycolytic activity of brain decreased rapidly with time, the oxidised dibenzanthracene was in contact with the tissue from the beginning in the glycolysis experiments. Though this lessens the accuracy of the determinations the effect found was so large as to be outside the possible error. The sections were dried either at 50° or at room temperature in a desiccator. Tables IX–XII show that the minimum concentration to show an effect is about 0.02 %.

B. *Malignant tissues.*

Jensen's rat sarcoma (J.R.S.), and C 63¹, a transplantable mammary carcinoma of the mouse, were used in this work. In addition three strains of transplantable tumours produced by pure hydrocarbons were studied, and their respiration and anaerobic glycolysis measured for the first time. These latter tumours which are spindle-celled sarcomas were produced here by subcutaneous injection of solutions in lard of 1:2:5:6-dibenzanthracene (tumour R 10, rat) or of 5:6-cyclopenteno-1:2-benzanthracene (tumour M 154, mouse), or of 1:2:5:6-dibenzanthracene in lard and milk (tumour M 4, mouse). The first of these has now reached the 51st, the second the 44th and the third the 83rd grafted generation.

Jensen's rat sarcoma. In addition to experiments similar to those described for other tissues, in a few cases considerable precautions were taken to avoid bacterial infection. The solutions used were all sterilised and measured out from sterile pipettes, the Warburg vessels and Petri dishes were autoclaved and the tumours removed aseptically from the rats. Sterile finger-stalls were used when cutting the sections. The gases were passed through cotton wool plugs, and the parts of the manometer through which they passed were rinsed out with absolute alcohol. Cultures were taken from the liquids at the end of the experiments and examinations made after 18 hours' incubation. Table XIV shows the result of one such experiment, with the bacteriological report.

¹ We are indebted to the Imperial Cancer Research Fund for this tumour.

Table XIII. *J.R.S. slices at 37.5°.*

Hour	Concentration of oxidised dibenzanthracene			
	0	0.1 %	0.2 %	0.3 %
	Q_{O_2} in Ringer-phosphate			
1	12.2	8.6	9.8	8.4
2	8.8	8.6	9.0	7.3
3	7.0	7.4	6.6	5.0
	$Q_M^{N_2}$ in Ringer-bicarbonate			
1	26.2	18.5	15.1	21.0
2	19.0	17.4	11.7	11.4
3	14.4	12.8	5.3	5.8

Table XIV. *J.R.S. slices at 37.5°, aseptic technique.*

(Roman numerals refer to bacteriological report.)

Hour	Q_{O_2} in Ringer-phosphate				
	Concentration of oxidised dibenzanthracene				
	No lactate				
	I	II	III	IV	V
		0	0.1 %	0.2 %	0.3 %
1	10.0	10.4	8.2	7.4	7.2
2	7.8	8.7	7.3	6.3	4.5
3	7.1	6.9	6.5	5.4	3.1
	$Q_M^{N_2}$ in Ringer-bicarbonate				
	Concentration of oxidised dibenzanthracene				
		VI	VII	VIII	IX
		0	0.1 %	0.2 %	0.3 %
1		19.2	14.0	15.0	9.5
2		14.0	11.1	11.4	6.1
3		8.0	5.7	4.6	4.1

Sterility test on fluid from experiment shown in Table XIV after 18 hours' incubation.

- Vessel I. Slight growth of streptococci and *B. subtilis*.
 Vessel II. Moderate growth of staphylococci and streptococci.
 Vessel III. Very slight growth of staphylococci.
 Vessel IV. Moderate growth of streptococci and staphylococci.
 Vessel V. Very slight growth of staphylococci.
 Vessel VI. Very slight growth of *B. subtilis*.
 Vessel VII. Sterile.
 Vessel VIII. Sterile.
 Vessel IX. Sterile.

Eighteen hours' incubation after the conclusion of the experiment constitutes a somewhat severe test of aseptic technique. It is evident in Table XIV that such degrees of contamination as had occurred show no parallelism with the chemical changes measured (*e.g.* vessels III, IV, V).

Dickens [1931] quotes results for J.R.S.:

Warburg, $Q_{O_2}=9$, $Q_M^{N_2}=34$;Crabtree, $Q_{O_2}=8$, 11, $Q_M^{N_2}=32$.

Rat sarcoma 10. Tables XV and XVI, typical of experiments on this tumour, show that concentrations of less than 0.1 % oxidised dibenzanthracene produce

no inhibition of oxygen uptake, within the times of the experiments (2-3 hours) and that higher concentrations (0.2, 0.3 %) show an effect only after one hour.

Table XV. *R. 10 slices in Ringer-phosphate at 37.5°.*

Hour	Q_{O_2}				
	12.5	12.2	12.6	13.0	13.2
	Concentration of oxidised dibenzanthracene				
	No lactate	0	0.017 %	0.033 %	0.05 %
2	12.2	12.2	12.6	11.5	13.5
3	11.1	11.7	10.7	10.7	12.5
	% difference, calculated on 1st hour				
2	-2	0	0	-12	+2
3	-11	-4	-15	-18	-5

Table XVI. *R. 10 slices in Ringer-phosphate at 37.5°.*

Hour	Q_{O_2}			
	13.8	15.0	12.7	13.8
	Concentration of oxidised dibenzanthracene			
	0	0.1 %	0.2 %	0.3 %
2	12.4	13.4	11.4	11.4
3	10.4	7.9	4.8	3.5
4	10.0	6.7	2.7	2.1
	% difference, calculated on 1st hour			
2	-11	-11	-10	-17
3	-25	-47	-62	-75
4	-28	-55	-80	-85

Table XVII. *R. 10 slices in Ringer-bicarbonate at 37.5°.*

Hour	$Q_M^{N_2}$			
	19.0	18.3	22.3	22.0
	Concentration of oxidised dibenzanthracene			
	0	0.1 %	0.2 %	0.3 %
2	14.5	13.8	12.0	10.0
3	11.6	11.7	9.2	5.7
4	6.2	7.1	6.2	4.2
	% difference, calculated on 1st hour			
2	-23	-25	-45	-55
3	-39	-35	-57	-74
4	-67	-61	-73	-82

Mouse sarcoma M 154. It was difficult to get sections of this tumour completely free from necrotic material, and as the method of calculation used makes use of the absolute value of the initial respiration of each sample, no great trouble was taken to ensure the absence of necrosis from the slices used. This accounts for the considerable differences in amount of respiration found. It will be seen that in the glycolysis experiments the differences in the first hour (after adding the oxidised dibenzanthracene) are greater than could reasonably be accounted for by the presence of some necrotic material.

Table XVIII. *M* 154 slices in Ringer-phosphate at 37.5°.

Hour	Q_{O_2}				
	10.0	9.8	9.5	8.4	8.0
	Concentration of oxidised dibenzanthracene				
	No lactate	0	0.017 %	0.033 %	0.05 %
2	7.3	9.6	7.8	8.0	7.4
3	5.8	7.5	6.5	6.3	5.0
4	3.7	6.7	5.4	5.4	4.3
5	3.1	5.8	5.1	4.7	2.6
	% difference, calculated on 1st hour				
2	-27	-2	-18	-5	-8
3	-42	-23	-31	-25	-38
4	-63	-32	-43	-35	-47
5	-69	-41	-46	-44	-68

Table XIX. *M* 154 slices at 37.5°.

Hour	Q_{O_2} in Ringer-phosphate			
	11.5	12.1	8.6	9.3
	Concentration of oxidised dibenzanthracene			
	0	0.1 %	0.2 %	0.3 %
2	11.0	9.7	5.2	1.8
3	9.1	8.4	2.8	1.5
	% difference, calculated on 1st hour			
2	-4	-20	-40	-81
3	-21	-30	-68	-84
	$Q_{M}^{N_2}$ in Ringer-bicarbonate			
1	21.5	12.8	10.6	7.8
2	18.4	12.2	8.3	6.2
3	17.4	10.3	6.7	5.7

Fig. 2. Glycolysis of *M* 154 slices in Ringer at 37.5°.

Mouse sarcoma M 4. Table XX shows one of three similar experiments on M 4. A concentration of 0.033 % produced an inhibitory effect within 3 hours and 0.05 % after one hour. It is thus rather more susceptible than R 10 and J.R.S.

Table XX. *M 4 slices in Ringer-phosphate at 37.5°.*

Hour	Q_{O_2}				
	9.0	10.6	9.5	9.0	10.5
Concentration of oxidised dibenzanthracene					
	No lactate	0	0.017 %	0.033 %	0.05 %
2	8.4	9.9	8.9	9.0	9.7
3	7.1	8.5	7.9	7.5	7.0
4	6.2	7.2	6.1	5.0	3.8
% difference, calculated on 1st hour					
2	-7	-6	-6	0	-8
3	-20	-20	-17	-17	-33
4	-31	-32	-36	-45	-64

Mouse carcinoma C 63. This tumour is vascular and has a small growing edge of sound tissue; sections were therefore difficult to cut free from necrotic material. This tumour appears to be rather more susceptible to the action of the oxidised dibenzanthracene than the others studied, as inhibition of respiration is observable after one hour even with a 0.033 % solution and 0.1 % produces almost the maximum inhibition (Tables XXI-XXIII).

Table XXI. *C 63 slices in Ringer-phosphate at 37.5°.*

Hour	Q_{O_2}				
	8.0	11.5	8.3	10.6	12.5
Concentration of oxidised dibenzanthracene					
	No lactate	0	0.017 %	0.033 %	0.05 %
2	6.7	10.5	7.2	7.2	8.0
3	5.4	8.2	6.6	6.4	6.7
4	3.7	6.8	4.7	4.5	4.8
% difference, calculated on 1st hour					
2	-16	-9	-13	-32	-36
3	-32	-29	-20	-40	-46
4	-54	-41	-43	-58	-62

Table XXII. *C 63 slices in Ringer-phosphate at 37.5°.*

Hour	Q_{O_2}				
	18.0	12.8	15.2	11.2	17.6
Concentration of oxidised dibenzanthracene					
	No lactate	0	0.1 %	0.2 %	0.3 %
2	10.2	8.6	8.0	5.3	7.5
3	7.4	5.5	4.8	2.8	3.8
% difference, calculated on 1st hour					
2	-43	-33	-47	-53	-56
3	-59	-57	-70	-74	-78

Table XXIII. *C 63 slices in Ringer-bicarbonate at 37.5°.*

Hour	$Q_M^{N_2}$			
	15.5	19.4	12.2	14.3
	Concentration of oxidised dibenzanthracene			
	0	0.1 %	0.2 %	0.3 %
2	18.2	16.1	8.4	8.5
3	15.7	10.2	6.4	5.2
	% difference, calculated on 1st hour			
2	+17	-17	-31	-41
3	+1	-47	-48	-63

CONCLUSION.

The tables given above show that oxidised 1:2:5:6-dibenzanthracene acts as an inhibitor of oxidation and glycolysis in all the tissues studied. There is no remarkable difference between the effects on normal and on malignant tissues, except that the malignant tissues with the exception of C 63 are more slowly inhibited. A concentration of 0.2 % in every tissue examined produces a measurable diminution of metabolism; in the case of kidney and muscle 0.02 % is effective. Boyland [1933], using the methylene blue technique, found that a 0.002 % solution of the hydrocarbon produced after oxidation more than 50 % inhibition of lactic dehydrogenase from yeast, but the quantity of this enzyme present was probably much smaller than in the present experiments.

As all known reactions involving enzymes take place in aqueous solution, it seems probable that carcinogenic hydrocarbons are altered in the body to form water-soluble compounds before they can produce biological effects. The oxidation products of these hydrocarbons produced by oxygen in ultra-violet light are water-soluble and have a large inhibitory effect on oxidation processes. The present work shows that the action of the oxidised 1:2:5:6-dibenzanthracene on malignant cells is very similar to that on normal tissues. It is possible that the production of tumours by these carcinogenic compounds involves an oxidation of the compound, either to a product identical with that isolated and used in the foregoing experiments, or to a less stable intermediate oxidation product. Subcutaneous injections of 0.4 % solutions of the oxidation products used in the present work have not yet, after 14 weeks, produced any tumours in rats or mice.

SUMMARY.

The effect of a water-soluble oxidation product of 1:2:5:6-dibenzanthracene on the metabolism has been studied in respect of the following rat tissues: 1. Muscle, respiration and glycolysis: 2. Liver, respiration: 3. Kidney, respiration: 4. Brain, respiration and glycolysis: 5. Jensen's sarcoma and rat sarcoma 10, respiration and glycolysis; and of the following mouse tissues: 6. Sarcoma M 154, respiration and glycolysis: 7. Sarcoma M 4, respiration: 8. Carcinoma 63, respiration and glycolysis.

In all cases an inhibitory effect was found.

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