LXXVI. EXPERIMENTS ON THE CHEMOTHERAPY OF CANCER

III. THE INDEPENDENCE OF TISSUE RESPIRATION AND GLYCOLYSIS, AND THE GROWTH RATE OF TUMOURS

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(Received 28 February 1939)

THE main activity of tumours appears to be growth and hence it might be expected that growth rate and respiration or glycolysis would be proportional to each other under different conditions since the energy used for growth processes is probably obtained from carbohydrate metabolism. The respiration or anaerobic glycolysis of a stimulated uninjured muscle is almost proportional to the work performed [Meyerhof, 1930]. Stimulation of isolated submaxillary gland tissue with acetylcholine, which increases secretion, also increases respiration [Deutsch & Raper, 1938]. Murphy & Hawkins [1925], however, found the respiration and glycolysis of spontaneous mouse tumours to be independent of rate of growth.

The inhibition of tumour growth in rats by irradiation with X-rays is not accompanied by marked changes in glycolysis until a very considerable degeneration of tissue has occurred [Bancroft *et al.* 1935]. Some reduction of respiration may occur: in the experiments on rats given by Bancroft *et al.* the reduction is significant in about half the treated animals. In experiments of Crabtree [1932], in which rats bearing the Jensen rat sarcoma were treated with radium, an increase in the anaerobic and aerobic glycolysis of the tumour tissue was sometimes produced and the respiration was generally reduced, but growth of the tumours continued. The inhibition of growth of tumours by radiation does not appear to be necessarily dependent on inhibition of glycolysis or respiration.

In the case of muscular activity it has been shown that the decomposition of creatine phosphate is a more fundamental or essential change for activity than respiration and glycolysis. The inhibition of glycolysis with iodoacetic acid does not completely inhibit muscular activity. It now seems probable that analogous fundamental processes may govern tumour growth as creatine phosphate breakdown limits muscular action. In the present paper it is shown that the effect of some tumour-growth-inhibiting substances does not change the respiration and glycolysis of tumour tissue. In the search for compounds likely to be useful for the chemotherapy of cancer it may be considered that effective substances need not necessarily affect these processes although they are the largest changes which occur in tumour cells. It is proposed to determine which cell constituents or biochemical processes vary with growth rate or are affected by treatment with the known tumour inhibitors. It is hoped that such results may give a rational basis for further experiments in therapy.

Growth rates and metabolism of different tumour strains. The growth rate of different strains of transplanted tumours in mice varies from 0.01 to 1.35 mm. per day, measured as increase in average diameter [Boyland & McClean, 1935, see Table I]. Values for the respiration (Q_{O_2} from -7.5 to -12) and glycolysis

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Table I. *Growth rates and tissue metabolism of different mouse tumours*

Tumour	Mean growth rate (mm. increase in diam. per day)	Respiration	Glycolysis
		Mean (min.-max.) - Q_{O_2}	Mean (min.-max.) $Q_L^{N_2}$
S 37	1.35	11.5 (8.5-13.1)	33.5 (23.3-39.2)
Crocker 180	0.88	8 (7.2- 9.4)	23 (20.5-26.2)
M 4	0.74	8.5 (7.6-10.9)	26 (22.1-34.0)
M.C.D.B.I.	0.70	10 (9.1-11.8)	28.5 (21.7-37.5)
Mal.	0.69	7.5 (7.2- 8.5)	25 (17.7-28.5)
C 63	0.66	12 (6.2-14.4)	25 (16.5-27.5)
L.M. 154	0.032	9.5 (8.0-10.2)	22 (20.2-25.6)
Melanoma (Passey)	0.010	8.5* (5.6-11.7)	16* (8.9-23.7)

* From Crabtree [1929].

($Q_L^{N_2}$ from 16 to 34), which were determined in the usual way in the Warburg apparatus at 37.5°, show much smaller variations which do not run parallel with growth rate. It should be remembered that the measurements of respiration and glycolysis of isolated tissues thus determined are measurements of ability to glycolyse and respire under artificial conditions and may be different from the rates of the tissues *in situ*. The rates of glycolysis observed probably represent the maximum values, but if either the ability to respire or glycolyse were the factors limiting growth they should vary with rate of growth of the tumour.

It would be of interest to determine whether glycolysis and respiration of different tumours growing in tissue culture correspond with the growth rate of the culture. This would allow determination of growth and metabolism under the same conditions, and then all determinations would be comparable.

Effect of treatment on tumour growth and metabolism. Two types of tumour were used. (a) Crocker sarcoma 180 grafted into mixed stock mice in the usual way. (b) Sarcoma M.C.D.B.I. originally obtained by subcutaneous injection of 1 mg. methylcholanthrene dissolved in 0.2 ml. olive oil into a male mouse of the pure Dilute Brown strain. The tumour has always been grafted into mice of the same strain, in which it grows readily and regularly: it is now in the eighth grafted generation. A tumour of this type, which has never grown outside the same pure line of mice, resembles spontaneous or induced tumours in that genetic differences between the tumour tissue and the host's tissues are probably unimportant. In the case of the Crocker sarcoma inhibition of growth on treatment may be due either to (1) increase in resistance of the host to a foreign tissue or (2) damage to the tumour tissue *per se*. In the case of the M.C.D.B.I. tumour the factor (1) should not operate. This latter tumour has some advantages over spontaneous tumours, viz. (a) it is more easily available in large numbers, (b) tumours in different mice grow at the same uniform rate so that some mice may be used as controls, (c) the chemical constituents and biochemical reactions are probably more constant.

Method. As soon after grafting as possible the size of tumours was measured with calipers every second day. Sodium sulphanilyl-sulphanilate and *pp'*-diaminodiphenylsulphoxide, the inhibiting action of which has been described [Boyland, 1938] and 4,4'-diamino-2,2'-dinitrodiphenylmethane and α -nitroso- β -naphthol, which have since been found to inhibit tumour growth, were administered by daily injection into the stomachs of mice, generally commencing on the day on which the tumours were grafted. The carcinogenic substances 1:2:5:6-dibenzanthracene and 1:2:5:6-dibenzfluorene, which Haddow *et al.* [1937] and Haddow [1938] have shown to inhibit tumour growth, were injected intraperitoneally as a single dose dissolved in oil about a fortnight after

grafting the tumour. Trypan Blue (as calcium salt) and Isamine Blue (as sodium salt), both of which stain tumour tissue, were injected intraperitoneally in four or five doses of 0.25 ml. of a 1 % aqueous solution, the first doses being given about a fortnight after grafting the tumours. After a suitable interval of treatment the mice were killed and the duplicate determinations of respiration and glycolysis of the tumour tissue were made in the Warburg apparatus. Occasionally the tumour growth was not inhibited; such tumours (except those in mice treated with Isamine Blue) were not used in the measurements.

Healthy tissue free from necrotic material was used for the determinations of metabolism. No obvious difference in the extent of necrosis in normal and inhibited tumours was observed, but closer investigation of this point will be made. Tumours inhibited by 1:2:5:6-dibenzanthracene or 1:2:5:6-dibenzfluorene often appeared to have a more fibrous structure than the controls and in these cases the fibrous material was necessarily included in the samples used for experiment.

Table II. *The effect of treatment on growth rates and tissue metabolism of mouse tumours*

Mouse treated with	Dose mg.	Mean inhibition of growth %	No. of mice	Respiration ($-Q_{O_2}$)			Glycolysis ($Q_L^{N_2}$)		
				mean ($\bar{x}$)	σ	$\frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(\epsilon_1^2 + \epsilon_2^2)}}$	mean ($\bar{x}$)	σ	$\frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(\epsilon_1^2 + \epsilon_2^2)}}$
Sarcoma M.C.D.B.I.									
Controls		—	(9)	10.5	± 1.7	—	29.6	± 5.2	—
1:2:5:6-Dibenzanthracene	15	100	(6)	10.0	± 1.2	<1	26.9	± 2.2	<1
1:2:5:6-Dibenzfluorene	5	66	(5)	7.6	± 2.4	1.4	26.6	± 0.9	1.8
Sodium sulphanilyl-sulphanilate	50	75	(6)	9.8	± 0.4	1.1	29.0	± 4.2	<1
<i>pp'</i> -Diaminodiphenyl-sulphoxide	8	50	(5)	9.5	± 1.2	1.3	23.0*	± 2.5	3.2
4:4'-Diamino-2:2'-dinitro-diphenyl-methane	8	30	(6)	10.3	± 0.8	<1	28.4	± 3.3	<1
α -Nitroso- β -naphthol	8	33	(5)	9.4	± 1.2	1.4	27.3	± 6.0	<1
Trypan Blue	2.5	83	(6)	6.3*	± 2.7	3.5	22.6*	± 4.7	2.7
Isamine Blue	2.5	0	(4)	10.3	± 0.4	<1	26.2	± 2.0	1.6
Crocker sarcoma 180									
Controls		—	(9)	8.1	± 0.7	—	22.8	± 2.1	—
1:2:5:6-Dibenzanthracene	15	66	(10)	7.8	± 1.5	<1	22.5	± 4.3	<1
1:2:5:6-Dibenzfluorene	5	60	(6)	7.5	± 1.4	<1	25.4	± 3.7	1.6
Sodium sulphanilyl-sulphanilate	50	50	(5)	8.3	± 0.7	<1	24.2	± 1.3	1.4
<i>pp'</i> -Diaminodiphenyl-sulphoxide	8	67	(7)	8.3	± 1.5	<1	25.0	± 2.5	1.8
4:4'-Diamino-2:2'-dinitro-diphenylmethane	8	45	(6)	9.0	± 1.1	1.8	24.2	± 2.2	<1
α -Nitroso- β -naphthol	8	50	(5)	7.6	± 1.5	<1	19.1	± 3.5	2.0
Trypan Blue	2.5	63	(7)	4.7*	± 1.6	5.3	13.2*	± 6.5	3.8
Isamine Blue	2.5	25	(5)	6.5	± 1.9	1.6	18.8	± 6.5	1.2

$\epsilon = \sqrt{\frac{\sum (\bar{x} - x)^2}{n(n-1)}}$ where $\bar{x}$ is the mean of the series of observations, x =any individual value and n =the number of observations. σ =standard deviation.

* These values are considered to be probably significantly different from the means for the corresponding control tumours—i.e. the values of $\frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}} > 2$, where $\bar{x}_1$ =mean value for controls, $\bar{x}_2$ =mean value of the series under consideration.

RESULTS

The results of this part of the experiment are given in Table II and show that with marked decrease in rates of growth, the metabolism is often not reduced. Significant reductions were found only (1) in the respiration and glycolysis of tumours in mice treated with Trypan Blue, and (2) in the glycolysis of M.C.D.B.I. from mice treated with *pp'*-diaminodiphenylsulphoxide.

SUMMARY

1. The glycolysis and respiration of several strains of grafted tumours with growth rates varying from 0.01 to 1.35 mm. per day are not proportional to the rate of growth and vary within a narrow range.

2. The values for glycolysis and respiration of tissue from grafted tumours, the growth of which was inhibited by administration of 1:2:5:6-dibenzanthracene, 1:2:5:6-dibenzfluorene, sodium sulphanilylsulphanilate, α -nitroso- β -naphthol and 4:4'-diamino-2:2'-dinitrodiphenylmethane, were not significantly lower than values of untreated tumours.

3. The inhibition of growth produced by Trypan Blue was accompanied by decrease in respiration and glycolysis while that produced by *pp'*-diaminodiphenylsulphoxide was accompanied by reduction in glycolysis only.

4. Injection of Isamine Blue did not inhibit tumour growth nor reduce the metabolism.

We should like to thank Dr Smith of the Wellcome Chemical Works for samples of sodium sulphanilylsulphanilate and *pp'*-diaminodiphenylsulphoxide, and Prof. J. W. Cook for a specimen of 1:2:5:6-dibenzfluorene.

We are indebted to the British Empire Cancer Campaign for financial support.

REFERENCES

- Bancroft, Beck & Russell (1935). *Biochem. J.* **29**, 2416.
Boyland (1938). *Biochem. J.* **32**, 1207.
— & McClean (1935). *J. Path. & Bact.* **41**, 553.
Crabtree (1929). *Biochem. J.* **23**, 536.
— (1932). *10th Sci. Rep. Imp. Cancer Res. Fund.*
Deutsch & Raper (1938). *J. Physiol.* **92**, 439.
Haddow (1938). *Brit. Emp. Cancer Campaign Rep.* **15**, 235.
— Scott & Scott (1937). *Proc. Roy. Soc. B*, **122**, 477.
Meyerhof (1930). *Die chemischen Vorgänge im Muskel*. Berlin: Springer.
Murphy & Hawkins (1925). *J. gen. Physiol.* **8**, 115.