CXLIII. THE METABOLISM OF NORMAL AND TUMOUR TISSUE.

II. THE RESPIRATORY QUOTIENT, AND THE RELATIONSHIP OF RESPIRATION TO GLYCOLYSIS[1].

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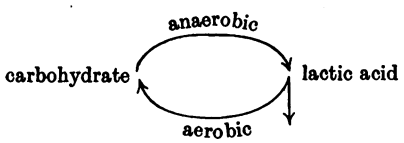
Recent progress in the study of gaseous exchange in tissues has led to many important discoveries, most of which have been derived from experiments upon isolated tissues. The data have been mainly concerned with the total value of respiration and, under aerobic and anaerobic conditions, the measurement of glycolysis. The relationship between these two processes forms one of the main problems of tissue metabolism, and is of very wide application.

Respiration has a definite effect on glycolysis (or fermentation) in almost all cases, from that of the yeast cell to the tissues of the higher animals. The relationship is not only qualitative, but the respiration of a definite amount of oxygen can only prevent the appearance of a related quantity of lactic acid. Meyerhof [1920, 1921] expresses the relationship in the form of the cyclical process:



In muscle, the quantitative aspect of this relationship has been worked out with considerable exactness. In other tissues the existence of some similar connection has been shown by the measurements of Warburg and of Meyerhof, and it is probable that a similar cycle, involving the resynthesis of lactic acid to carbohydrate occurs in tissues other than muscle. The original view of the workers on muscle, that the whole cycle is concerned with carbohydrate has not yet been definitely proved or disproved [Meyerhof and Himwich, 1924]. In the case of other tissues there is a great need for more evidence on the nature of the cycle, the extent to which it concerns carbohydrate and how far

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the energy required for resynthesis may be provided by the oxidation of other foodstuffs.

If the facts relating to this point are examined, the difficulty is encountered that evidence is largely lacking as to the relative amounts of the various foodstuffs which are consumed by individual body tissues. The direct chemical determination is usually a matter of great difficulty, and measurement of the respiratory quotient is the only practicable method of attacking this question.

When the metabolism of the tumour cell is considered, special difficulties arise, many of which are connected with the above facts. In tumours, just as in normal tissues, the respiration of a definite quantity of oxygen can prevent the appearance of only a certain amount of lactic acid. According to Warburg [1926, p. 139] the Meyerhof quotient, anaerobic glycolysis *minus* aerobic glycolysis/respiration, has the value 1–2 in carcinoma tissue, lactic acid bacteria, embryonic tissue and a number of other glycolysing tissues. This means that 1 mol. of breathed oxygen causes the disappearance of 1–2 mols. of lactic acid, and therefore in this sense the respiration of the cancer cell is as effective as that of muscle in preventing the appearance of aerobic glycolysis. Since tumour tissues does, in fact, produce much lactic acid aerobically, Warburg's conclusion was that the extent of respiration in tumours is too small to prevent aerobic glycolysis. This view, although apparently adequate in the earlier examples studied (*e.g.* Flexner Jobling rat carcinoma) is no longer tenable. Warburg [1929] now distinguishes another type of damage to respiration, in which the efficiency is impaired in some way, so that although the respiration may be large it is unable to prevent the appearance of lactic acid. The analogy is emphasised beteen the latter state and the poisoning of tissues, *e.g.* by ethyl *iso*cyanide, in which condition the extent of the respiration is stated to be unaffected but the aerobic glycolysis is the same as in nitrogen [Warburg, 1926]. Ethyl *iso*cyanide is therefore said to be a specific inhibitor of the Pasteur reaction, *i.e.* of the relationship between respiration and fermentation, using the latter term to include glycolysis. It is assumed that similar interference with this relationship occurs in tumours, and there are on this theory two abnormalities in the respiration of tumours: (*a*) damage limiting the extent of respiration (*i.e.* partial asphyxiation), and (*b*) damage to the Pasteur reaction, so that although the respiration is large the glycolysis persists.

In the present paper evidence is brought forward which reduces the complexity of the above assumptions, and indicates more clearly the actual nature of the damage to respiration in tumours. These facts have been gained from a study of the R.Q. of a series of normal organs, embryonic tissues and tumours.

There are other problems where the study of the R.Q. of animal tissues is of great value. For example, the action of fluoride in inhibiting anaerobic glycolysis [Dickens and Šimer, 1929] suggested that this substance might have some similar action on the oxidative catabolism of carbohydrate. In cases such as this, measurement of the total respiration without measurement of

the R.Q. is of little use. Moreover the measurement of R.Q. provides one of the most valuable methods of studying the intermediary stages in oxidation. This is the only satisfactory method of approach to the actual point of the damage in tumour respiration, and one that had hitherto been largely neglected mainly owing to the lack of suitable experimental methods. The method already described [Dickens and Šimer, 1930] is applied to some aspects of these problems in the present paper.

Metabolism of normal tissues. The special difficulties connected with the metabolism of tumours cannot be solved without first making similar observations on a series of normal tissues, since only in this way can any differences between the two be revealed. The observations on tumours and normal tissues form the subject of the present paper. In addition, it is necessary to investigate types of pathological growth other than tumours, in order to see how far the abnormalities found are restricted to tumour tissue, or if they are associated with other types of pathological growth. This will form the subject of a further part of this series.

No systematic attempt appears to have been made to measure the R.Q. of a representative series of normal tissues. The valuable recent observations of Richardson, Shorr and Loebel [1930] have been mainly from the special point of view of the problem of carbohydrate metabolism in diabetes. Other reliable measurements on tissue other than muscle and nerve are relatively few, and are quoted under the tissues concerned in the discussion of the experimental results. In this paper the values for muscle and nerve have not been considered, and no measurements have been made on these tissues, since in them the metabolism is very intimately connected with the special functions and their resting metabolism is of little value if considered apart from the working metabolism.

Experimental.

The method used for the determination of the R.Q. has been fully described in Part I [Dickens and Šimer, 1930]. The tissue is cut into thin slices and suspended in Ringer solution, to which have been added a suitable amount of phosphate buffer at p_H 7·4, and sufficient glucose to make the concentration of the latter 0·2 %. The measurement is made in special vessels filled with pure oxygen and attached to Barcroft manometers, from readings of which both the oxygen consumed and the carbon dioxide evolved are calculated.

Regularity of respiration in the phosphate-Ringer solution. The oxygen consumption in unit time is as constant in the above medium as in the bicarbonate-Ringer or serum customarily used, if glucose or some substitute for glucose is present in the medium [Loebel, 1925]. In the absence of glucose it is necessary to take into account the increased nitrogenous catabolism, which may usually be neglected in the presence of glucose. Nearly all our measurements have been made in a solution containing glucose. There is also a quantitative agreement in the different media, as may be seen from the examples in Table I. In this table all the figures for the extent of respiration in phosphate are the

mean of all our experiments, most of which extended over a period of 2–3 hours or more. The figures are not, therefore, maximal values; nevertheless the agreement is satisfactory, showing that the respiration remains nearly constant for some hours.

Table I.

Tissue*	Respiration in phosphate-Ringer (mm.^{3} O_2 per mg. tissue per hour) Q_{O_2}	Respiration in bicarbonate-Ringer [Warburg, 1927] Q_{O_2}
Kidney	19·9	21
Intestinal mucosa ...	9·4	12
Testis	9·8	12
Brain cortex	12·4	11
Spleen	9·9	12
Jensen sarcoma ...	10·6	9
Rous chicken sarcoma	4·2	5

* Of rat unless stated.

Table II.

Tissue	Specimen no.	R.Q.	Tissue	Specimen no.	R.Q.
Liver	5	0·83 0·81	Chicken embryo	1	1·01 0·99
	7	0·86 0·87	Retina	1	0·99 1·00
Kidney	5	0·84 0·86	Jensen sarcoma	3	0·83 0·85 0·82
Spleen	2	0·88 0·90 0·90	Rous chicken sarcoma	2	0·93 0·92 0·90 0·91
Brain cortex	3	1·00 1·01	Spontaneous mouse tumour	1	0·91 0·91 0·91

In Table II the figures bracketed under any particular specimen number, were all obtained in the same experiment on slices from the same piece of tissue, except in the case of the embryo and retina, where the material from a single specimen was insufficient. The agreement in the values of R.Q. in the last column of this table is sufficient confirmation that the accuracy is in agreement with that to be expected from the considerations given in Part I. Even in the case of the embryo, where owing to the large amount of preformed CO_2 (about 70 mm.^{3} in the particular example quoted) the correction is much larger than usual, the agreement has been satisfactory in all experiments.

Only in a few of the earlier experiments where the volumes of gas measured were insufficient, was the error slightly in excess of the above limit.

The respiratory quotient of normal tissues. The experimental animals used for the determination of the R.Q. of normal tissues were rats in a normal state of nutrition unless otherwise stated, and were mainly derived from the Glaxo stock. Their age varied from a few months to about a year. In the following

description of the results, the tissues are classified into three groups, according to the value of R.Q. observed in each case.

A. Tissues with a low value of R.Q.

(*a*) *Kidney.* Thin slices were cut through the whole organ and included both medulla and cortex. The results of nine experiments are collected in Table III, in which the volumes of oxygen consumed and carbon dioxide formed in the stated time are given. As with all the figures given in this paper, the weights of the tissue are those obtained after drying to constant weight at 110°. These results are typical of those obtained in all experiments.

Table III. *Rat kidney.*

38°. In Ringer solution with 0·2 % glucose and 9·9 m.-mols. phosphate per litre.

Dried weight of tissue mg.	Time hr.	Time min.	O_2 consumed mm.³	CO_2 produced mm.³	R.Q.	Respiration Q_{O_2} (whole exp.) mm.³/mg. hr.
6·23	2	0	215	164	0·76	17·3
6·40	2	0	238	185	0·78	18·6
4·99	2	0	215	194	0·90	21·8
6·83	2	0	322	297	0·92	23·5
3·54	2	0	132	109	0·83	18·7
15·88	1	15	394	331	0·84	19·8
14·69	1	15	405	360	0·89	19·6
12·15	1	45	404	349	0·86	19·0
18·73	1	0	384	353	0·92	20·5
Mean					0·85	19·9

From Table III it will be seen that the average value of the R.Q. is a little higher than in the case of liver (Table IV), although both are much below the carbohydrate level.

Table IV. *Normal tissues.*

38°. In phosphate-Ringer solution at p_H 7·4 with 0·2 % glucose.

	No. of experiments	Concentration of phosphate m.-mols. per litre	Highest R.Q.	Lowest R.Q.	Mean R.Q.
A. Low R.Q.					
(*a*) Rat kidney	9	9·9	0·92	0·76	0·85
(*b*) Rat liver	14	9·9	0·87	0·69	0·79
(*c*) Rat intestinal mucosa ...	2	9·9	0·85	0·84	0·85
B. Intermediate R.Q.					
(*a*) Rat submaxillary	5	9·9	0·92	0·83	0·87
(*b*) Rat spleen	5	9·9	0·91	0·87	0·89
(*c*) Rat testis	6	9·9	1·00	0·90	0·94
C. Carbohydrate R.Q.					
(*a*) Rat brain cortex	6	9·9	1·01	0·96	0·99
(*b*) Embryo:					
i. Rat: wt. 10 mg.	2	18·2	1·05	1·04	1·04
ii. Rat: wt. 30 mg.	2	18·2	1·07	1·01	
iii. Chicken (Table V: at 41°)	2	18·2	1·01	0·99	1·00
(*c*) Rat chorion	1	31·2	—	—	1·02
(*d*) Rat retina	2	9·9	0·99	1·00	1·00

(*b*) *Liver.* The results are summarised in Table IV. In this Table only the highest, lowest and mean values of the R.Q. are included, in order to economise space. The individual figures varied very similarly to those given in Table III for kidney. No special precautions were taken in the experiments to select all the slices from the same lobe of the liver, and the period since the animals were last fed was also variable, but in no case exceeded 24 hours. The variations in the results far exceed the error of the method, and will be discussed later.

(*c*) *Intestinal mucosa.* The mucous membrane was scraped with a blunt instrument from the muscular coat of the washed ileum of the rat. One specimen only was examined. (R.Q. = 0·85: see Table IV.)

B. Tissues with intermediate value of R.Q.

(*a*) *Submaxillary.* The rat submaxillary is well suited for these experiments and measurements of its anaerobic glycolysis ($Q_{CO_2}^{N_2} = 7$, in nitrogen and bicarbonate-Ringer solution) were made since no figure was available for this tissue in the rat. In Table IV the measurements of R.Q. are summarised. The mean value of Q_{O_2} found in the five experiments was 11·9, and of R.Q. 0·87.

(*b*) *Spleen* (see Table IV). This tissue gave very regular values of R.Q. in all experiments, the mean value being 0·89, and the mean of Q_{O_2} in the same series = 9·9.

(*c*) *Testis.* In the case of rat testis, the tubules are sufficiently thin to make the further cutting up of the material after dissecting unnecessary. The results of the measurements are given in Table IV.

C. Tissues with a carbohydrate quotient.

(*a*) *Brain cortex.* The grey matter of the rat's brain was carefully freed from the white substance. Thin slices were then cut with the razor as usual. From Table IV it is evident that the respiratory quotient is, within the limits of error, equal to unity.

(*b*) *Embryo.* (i) *Rat embryo.* Two sets of experiments were made with embryo at different periods of development. In the first the embryo weight was about 10 mg. (dried at 110°) and in the second about 30 mg. The embryos were not cut up in any way, but were merely freed from their foetal membranes and used intact. Owing to the thickness of the tissue the diffusion is necessarily poor and the respiration somewhat low. For the same reason, the amount of preformed CO_2 was large. Consequently, the values of R.Q. are less accurate in this case than in the other tissues studied, but the agreement is fairly satisfactory and indicates a carbohydrate quotient (Table IV).

(ii) *Chicken embryo.* The embryos were carefully removed from the eggs on the 5th day of incubation, and were thoroughly rinsed in oxygenated phosphate-Ringer before use. The suitability of the phosphate-Ringer as a medium is well shown by the fact that the respiration continued with only a slight diminution in the case of this delicate tissue for the whole of the experimental

period of 3½ hours. (See Table V. Details of the method of calculation are given in Part I.)

Table V. *Chicken embryo.*

41°. 5th day of incubation. Ringer solution with 0·2 % glucose and 18·2 m.-mols. phosphate per litre.

Vessel no.	II	III	IV	V	I	VI
	Each vessel contains solutions and 1 embryo				Solutions only	
Determination of preformed CO_2: pressure on acidification (mm.) ...	—	+39	—	+35	+ 8·5	+ 9
Measurement of respiration: pressure changes per 30 min. (mm.) ...	- 36	—	- 39	—	—	—
	- 34	—	- 37	—	—	—
	- 33·5	—	- 38·5	—	—	—
	- 32·5	—	- 37	—	—	—
	- 32	—	- 36·5	—	—	—
	- 30·5	—	- 34·5	—	—	—
	- 29	—	- 35·5	—	—	—
Total pressure change in 3½ hr. (mm.)	-227·5	—	-258	—	—	—
Pressure due to total CO_2 liberated at end of experiment	+256·5	—	+286·5	—	—	—
Embryo wt. (dried at 110°, mg.) ...	11·49	10·61	13·98	9·57	—	—
Vessel constants k_{O_2}	1·68	—	1·51	—	—	—
Vessel constants k_{CO_2}	1·81	1·87	1·64	1·80	1·65	1·74
Respiration O_2 (mm.³)	382	—	388	—	—	—
Total CO_2 liberated (mm.³) ...	464	73	469	63	14·1	15·6
					Mean 15	
Correction (preformed and solutions) (mm.³)	78	—	85	—	—	—
Respiration CO_2 (mm.³)	386	—	384	—	—	—
Q_{O_2} (oxygen per mg. tissue per hr.)	9·5	—	7·9	—	—	—
Respiratory quotient	1·01	—	0·99	—	—	—

(*c*) *Chorion.* The case of chorion is of special interest since the properties of growth and invasion possessed by the chorion have frequently been compared with the similar properties of tumours. Chorion has the highest anaerobic glycolysis of any normal body tissue, except retina [Negelein, 1925]. The material was the outer membrane of the amniotic sac of rat-embryos of about 30 mg. dry-weight. The result of an experiment is given in Table IV, and the figures obtained (8·04 mg. dry weight chorion, in 2 hours consumed 214 mm.³ O_2 and produced 219 mm.³ CO_2; R.Q. = 1·02) show that the quotient is a carbohydrate one.

(*d*) *Retina.* The preparation of the material was carried out as described by Warburg [1926] in the dimmest possible light, and in warmed phosphate-

Table VI. *Retina of rat.*

38°. In Ringer solution with 0·2 % glucose and 9·9 m.-mols. phosphate per litre.

Dried weight of tissue mg.	Time hr.	Time min.	O_2 consumed mm.³	CO_2 produced mm.³	R.Q.
2·68	5	0	182	180	0·99
2·69	5	0	159	159	1·00

Ringer solution. Also the vessels, each of which contained the retinas from two rats, were protected from direct light throughout. During this necessarily prolonged experiment, the respiration diminished considerably, but the result was nevertheless quite definite, and showed that the retina has a carbohydrate quotient (Table VI).

Respiratory quotient of tumour tissue.

The animal material used consisted of two types of rat tumour, Rous chicken sarcoma, and a number of mouse tumours. The specimens were obtained from the Barnato-Joel Laboratories and Bland-Sutton Institute of Physiology, Middlesex Hospital, and the Imperial Cancer Research Fund Laboratories respectively. Two suitable specimens of spontaneous mouse tumour were obtained from the breeders. For suitable human material we are indebted to the surgeons of the Middlesex Hospital.

Jensen rat sarcoma. Effect of phosphate concentration. The measurements were made in two different concentrations of phosphate buffer at p_H 7·4, and the results are collected in Table VII.

Table VII. *Jensen rat sarcoma.*

38°. In Ringer solution with 0·2 % glucose.

Dried weight of tissue mg.	Time hr.	min.	O_2 consumed mm.³	CO_2 produced mm.³	R.Q.	Respiration Q_{O_2} (whole exp.) mm.³/mg. hr.
9·9 m.-mols. phosphate per litre:						
19·69	1	15	269	216	0·80	10·9
18·20	1	30	303	269	0·89	11·1
26·54	1	30	384	336	0·88	9·7
13·84	2	0	326	272	0·84	11·8
17·58	2	0	373	304	0·82	10·6
17·65	2	0	380	319	0·84	10·8
4·69	5	0	294	244	0·83	12·5
5·62	5	0	340	290	0·85	12·1
4·70	5	0	274	224	0·82	11·7
Mean					0·84	11·2
31·2 m.-mols. phosphate per litre:						
21·68	1	30	369	300	0·81	11·3
14·53	2	0	328	267	0·82	11·1
29·14	1	30	375	317	0·85	8·6
18·67	2	15	414	358	0·87	9·9
25·78	1	45	461	360	0·78	10·2
24·94	2	0	526	418	0·80	10·5
29·94	1	30	422	328	0·78	9·4
17·79	1	45	310	235	0·76	10·0
Mean					0·81	10·1

The mean values obtained in the two series of experiments do not differ appreciably, so that the influence on the values of R.Q. and Q_{O_2} of varying the phosphate concentration, and consequently the buffering power, between these limits can be neglected. The actual value of R.Q. is comparable with that found for feebly glycolysing normal tissues.

Variation of p_H and glucose concentration, using tissues which glycolyse aerobically.

It was at first considered possible that the low R.Q. of tumour tissue might be due to an extreme sensitiveness of carbohydrate oxidation to slight changes in the reaction of the medium, such as occur with tissues showing a high aerobic glycolysis. Two sets of observations already recorded made this assumption unlikely: (*a*) the above experiments with weak and strong solutions of phosphate, in which nearly identical values of R.Q. and respiration were obtained, and (*b*) the carbohydrate quotient obtained with retina, a normal tissue with high aerobic glycolysis. Further evidence on this important point was obtained by the measurement in an acid medium of the R.Q. and respiration of rat brain-cortex which has a purely carbohydrate metabolism under the usual conditions of our experiments, *i.e.* in glucose and phosphate at p_H 7·4.

Accordingly, a CO_2-free isotonic phosphate buffer at p_H 6·6 was prepared and was used instead of the usual buffer in preparing the phosphate-Ringer solution. This p_H is considerably lower than that found even in extreme cases of glycolysing tissues, as is shown in the next section. The other conditions were identical with the experiments on brain recorded in Table IV. The respiration of brain tissue continued in this acid solution for 3 hours with only very slight falling off in the readings, and the values of the R.Q. and the Q_{O_2} were, within the limits of error, identical with those previously determined at p_H 7·4.

Condition of the medium in tumour experiments. The principal changes to be expected with glycolysing tissues are a lowering of the glucose content to an extent much greater than that due to respiration alone, and a change in the reaction of the medium towards the acid side, due to the large quantities of lactic acid formed from glucose. Jensen sarcoma was selected as an example of a tissue with a high aerobic glycolysis and a fairly low respiration. The conditions of the experiments were exactly as in the measurement of R.Q. (Table VIII). As an extreme case, the whole range of the manometer capillary

Table VIII. *Jensen rat sarcoma. Aerobic glycolysis in phosphate-Ringer.*

38°. In Ringer solution with 0·2 % glucose and 31·2 m.-mols. total phosphate.

Volume of phosphate-Ringer	2 cc.
Duration of experiment	2 hr. 10 min.
O_2 consumed	475 mm.^{3}
Dried weight of tissue	23·91 mg.
p_H—initial	7·41
p_H—final	6·79

Concentration of phosphate (m.-mols. per litre):

Na_2HPO_4—initial	25·4	final*	*ca.* 15·6
NaH_2PO_4—initial	5·8	final	*ca.* 15·6

Hence concentration of lactate formed = 25·4 − 15·6 = *ca.* 10 m.-mols. per litre.

$Q^{O_2}_{CO_2}$ (aerobic glycolysis in phosphate; p_H 7·4 to 6·8) = 9 approx.

„ (measured at the same time in bicarbonate-Ringer, p_H 7·4) = 14.

* Since final p_H, in this experiment, is nearly equal to p_K for phosphoric acid.

was used in this experiment, and a small additional correction was applied for the respiration during the period of temperature equalisation preceding the first reading.

From this it is evident that glycolysis occurs quite regularly in the phosphate medium, and that even in this extreme case the buffering is sufficient to keep the reaction considerably nearer neutrality than in the experiment with rat brain at p_H 6·6 quoted above. It may also be calculated from this experiment that the average glucose concentration during the whole experiment is about 0·15 %, there is thus no lack of glucose in the medium in any of the experiments recorded.

Table IX. *Tumour tissue.*

38°. In phosphate-Ringer solution at p_H 7·4 with 0·2 % glucose.

	No. of experiments	Concentration of phosphate m.-mols. per litre	Highest R.Q.	Lowest R.Q.	Mean R.Q.
Animal tumours:					
Jensen rat sarcoma	9	9·9	0·89	0·80	0·84
Jensen rat sarcoma	8	31·2	0·87	0·76	0·81
Slow-growing rat sarcoma ...	4	25	0·97	0·93	0·94
Rous chicken sarcoma ...	4	25	0·93	0·90	0·92
Rous chicken sarcoma at 41°	2	25	0·95	0·94	0·95
Transplanted mouse tumours:					
Spindle-celled tar tumour 173	2	25	0·91	0·90	0·91
Tar carcinoma 2146	2	25	0·89	0·85	0·87
Crocker sarcoma	2	25	0·90	0·87	0·89
Sarcoma 37 S	3	25	0·89	0·82	0·86
Spontaneous mouse tumours:					
Carcinoma I	3	25	0·91	0·91	0·91
Mammary carcinoma II ...	1	25	—	—	0·87
Human tumours:					
Papillary bladder carcinoma	4	25	0·91	0·78	0·86
Carcinoma of breast	3	25	0·86	0·81	0·83

Slow-growing rat sarcoma.

Although the quotient for this tumour (Table IX) is higher than in the case of Jensen sarcoma it is still quite definitely below the carbohydrate level. The value for anaerobic glycolysis obtained by the bicarbonate method was $Q^{N_2}_{CO_2} = 18$.

Rous chicken sarcoma. The results are given in Table IX. The variability in respiration observed was similar to that shown in the measurements of Crabtree [1928]. The value of R.Q., however, is fairly constant, the extreme measurements being from 0·90 to 0·95.

Mouse tumours. The group of mouse tumours provides examples of many exceptions to attempts to classify animal tissues according to their metabolism [Murphy and Hawkins, 1925; Crabtree, 1928; Warburg, 1927, 1929]. The specimens of transplanted mouse tumour used were supplied to us through the courtesy of Prof. J. A. Murray, F.R.S., and the metabolism of these

particular strains has been carefully studied by Crabtree [1929]. The results obtained in the measurement of R.Q. are given in Table IX. The results of simultaneous measurements of their metabolism in bicarbonate-Ringer solution [Warburg, 1926] are given below (Table X).

Table X.

	Respiration Q_{O_2}	Aerobic glycolysis $Q_{CO_2}^{O_2}$	Anaerobic glycolysis $Q_{CO_2}^{N_2}$
Spindle-celled tar tumour 173 ...	3·4	11·0	21·0
Tar carcinoma 2146	6·9	13·4	22·5
Crocker sarcoma	—	—	21·8
Sarcoma 37 S	6·5	19·9	26·6
Spontaneous mouse carcinoma I ...	7·5	8·1	20·1
Spontaneous mouse carcinoma II ...	11·3	8·8	16·0
Papillary bladder carcinoma, S.S. 365	1·5	3·1	3·4
Carcinoma of breast, S.S. 371 ...	1·7	2·9	7·1

Spontaneous mouse tumours. Two specimens were examined. The first was a columnar-celled carcinoma of high cellularity. The second was a mammary carcinoma with about 50 % connective tissue. The measurements of R.Q. are recorded in Table IX, and of the metabolism in bicarbonate-Ringer in Table X.

The values of R.Q. found for the mouse tumours are in no way different from those obtained with the transplanted rat tumours.

Human tumours.

Up to the present only two suitable cases of human tumours have been studied. The first was a papillary carcinoma of the bladder, Bland-Sutton report No. SS. 365/1930. Histologically this was a non-keratinising, squamous-celled carcinoma, invading muscle. The mixed nature of the tissue was shown not only by the variation in the value of respiration with different pieces, but also by the variation in the figures for R.Q. The second case (No. SS. 371/1930) was a spheroidal-celled carcinoma in a fibrous stroma. The values for respiration observed in bicarbonate-Ringer solution (Table X) agree with the mean values of the experiments in phosphate-Ringer ($Q_{O_2} = 1{\cdot}5$ and 2·5 respectively). The values observed for aerobic and anaerobic glycolysis are also given in Table X. Both tissues had a low respiration and contained much connective tissue. In spite of the prolonged experiment and large pieces necessary in these two cases, the results are in agreement with the other tumours examined in showing a quotient distinctly below unity.

Human tuberculous lymphatic gland. One specimen of tuberculous lymphatic gland was examined (Table XI). For this very suitable material and for the following report, we are indebted to Mr David Patey, F.R.C.S.

Macroscopical appearance. The swelling was smooth and rounded, and approximately 3 cm. in diameter. On section, it presented a uniform appearance without obvious necrosis.

Table XI. *Human tuberculous lymphatic gland.*

Dried wt. of tissue mg.	Time hr.	Time min.	O_2 consumed mm.³	CO_2 produced mm.³	R.Q.	Q_{O_2}	Metabolism in bicarbonate-Ringer: Respiration Q_{O_2}	Aerobic glycolysis $Q^{O_2}_{CO_2}$	Anaerobic glycolysis $Q^{N_2}_{CO_2}$
38·6	1	45	396	365	0·92	5·8	5·5	8·2	14·2
33·1	2	0	391	354	0·91	5·9			
30·8	2	0	371	336	0·91	6·0			
40·5	1	30	426	387	0·91	7·0			
				Mean	0·91	6·2			

Microscopical appearance. This showed the typical appearance of a tuberculous lymphatic gland (Plate V, Figs. 1 and 2). On a lymphoid stroma were large areas of endothelial cell proliferation, occasional giant cell formation and small areas of caseation.

Effect of lactate and pyruvate.

From the point of view of intermediary metabolism the effect of lactate and pyruvate on the R.Q. is of special interest. Characteristic tissues from the above three groups were chosen. These were (1) liver, (2) testis, and (3) tumour tissue. The solutions used were the usual phosphate-Ringer with or without 0·2 % glucose, and the same solution with the glucose replaced by sodium lactate or sodium pyruvate respectively, in amount sufficient to make the final concentration of these substances $M/45$. The results of the measurements are reproduced in Table XII.

Table XII.

Tissue	Exp. no.	R.Q.: Glucose 0·2 %	R.Q.: Lactate $M/45$	R.Q.: Pyruvate $M/45$	Q_{O_2}: Glucose 0·2 %	Q_{O_2}: Lactate $M/45$	Q_{O_2}: Pyruvate $M/45$
Liver	1	0·83	0·88	—	6·4	8·1	—
		0·81	0·87	—	6·6	8·9	—
	2	0·76	0·84	1·20	7·6	9·0	7·5
				No addition			No addition
Testis	1	—	0·91	0·76	—	6·4	4·4
		—	0·89	0·75	—	6·5	4·8
	2	0·94	—	—	9·8	—	—

(Figures in glucose are mean value of previous experiments in case of testis.)

				Pyruvate			Pyruvate
Jensen rat sarcoma	1	0·87	0·85	1·05	9·9	12·2	11·8
Rous chicken sarcoma	1	0·94	—	1·17	6·3	—	7·2
		0·95	—	—	5·9	—	—

More reliance can be placed on the values of R.Q. than on the actual extent of respiration in these experiments. The changes in the value of R.Q. are quite definite, whereas a series of experiments would be necessary to obtain quantitative evidence of the effect on the respiration. From the figures in this table it will be seen that in both experiments with liver there is a definitely higher

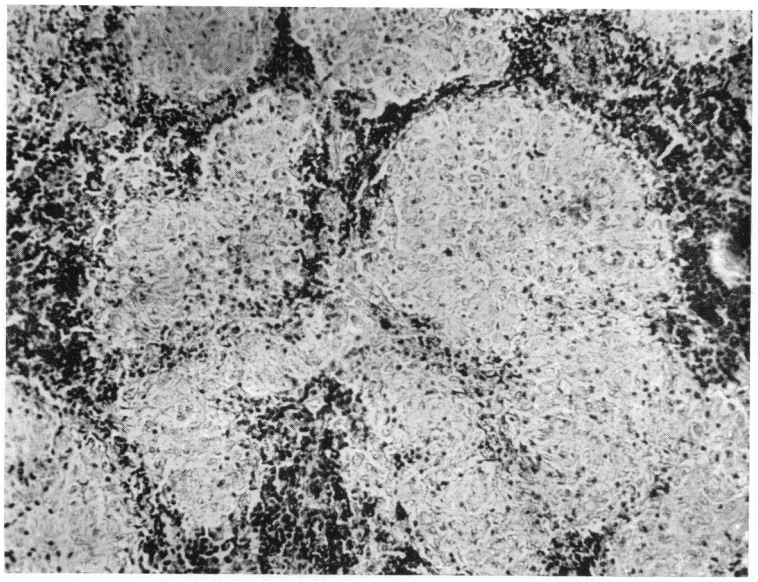


Fig. 1. Low power view of tuberculous gland showing large areas of endothelial cell proliferation in a lymphoid stroma. × 75.

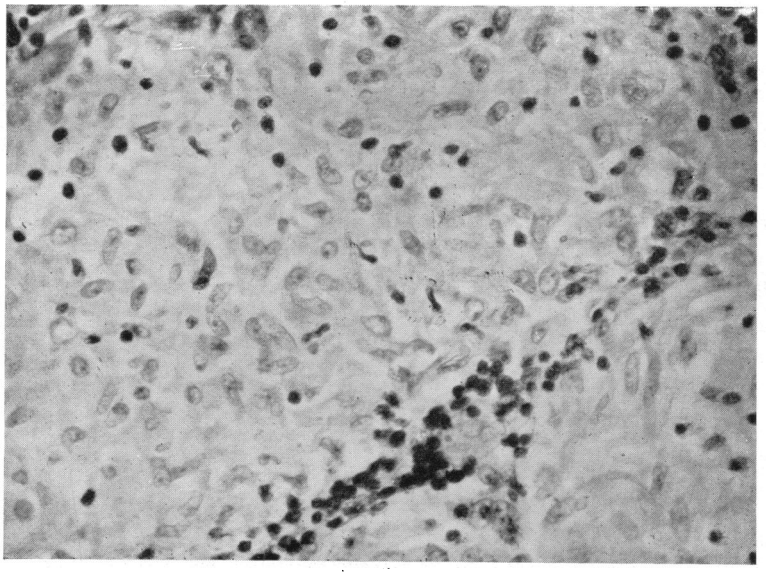


Fig. 2. High power view of an area of proliferated endothelial cells. × 390.

R.Q. in lactate than in glucose, in addition to the increased extent of respiration. The latter has been previously described by Meyerhof and Lohmann [1926]. From the figures for testis a similar utilisation of lactate is observed. The value in lactate is similar to the average figure obtained in glucose, whilst a much lower value of respiration and of R.Q. is obtained in the absence of either glucose or lactate. Turning to the case of the tumour tissue, on the other hand, in the example of the Jensen sarcoma there is no effect on the R.Q. if lactate is added to the medium instead of glucose. There is thus an important distinction between the effect of lactate on the respiration of normal and tumour tissue. The significance of these results will be considered later.

The effect of pyruvate is even more striking. The value of R.Q. in all the above tissues, normal or tumour, was much increased by addition of pyruvate. The values obtained with liver even reached the theoretical value for complete oxidation of pyruvic acid (R.Q. = 1·20); it should however be noted that Meyerhof, Lohmann and Meier [1925] calculate for oxidative resynthesis of pyruvate to carbohydrate the probable value R.Q. = 2. Large increases in the value of R.Q. were also observed in the above experiments with Jensen sarcoma and Rous sarcoma.

Effect of fluoride. The action of fluoride in inhibiting anaerobic glycolysis has formed the subject of an earlier paper [Dickens and Šimer, 1929] where it was shown that the action was quantitatively reproducible and that the effect of different concentrations followed the law of mass action. Since fluoride has little effect on the formation of lactic acid from methylglyoxal [Meyerhof, 1925], it follows that the point of attack of fluoride must be at an earlier stage in the intermediary metabolism of carbohydrate. It is therefore of importance to determine whether the oxidative catabolism of carbohydrate is affected by addition of fluoride.

Two types of tissue were studied, (1) kidney, and (2) testis. These measurements (Table XIII) have to be made in Ca-free phosphate-Ringer solution, to avoid difficulties due to the precipitation of CaF_2.

Table XIII. *Effect of NaF on rat kidney and testis.*

In Ringer solution without Ca, and 9·9 m.-mols. phosphate in all.
Concentration of fluoride: 20 m.-mols.

	Specimen no.	R.Q. Without NaF	R.Q. With NaF	Q_{O_2} Without NaF	Q_{O_2} With NaF
Kidney 0·2 % glucose	1	0·91	—	13·1	—
	2	0·83	—	16·7	—
		0·85	—	13·8	—
	3	—	0·83	—	7·6
		—	0·83	—	7·1
	4	0·86	0·71	15·8	10·5
Testis 0·2 % glucose	1	—	0·87	—	4·5
	2	—	0·81	—	3·1
M/45 lactate	1	—	0·91	—	6·5
	2	—	0·89	—	5·8

From the above figures it will be seen that the effect of fluoride on kidney on the whole tends to lower the value of R.Q. and hence probably has at least a slight inhibitory effect on carbohydrate oxidation as well as on glycolysis. In this tissue, however, the effect is not particularly striking, probably because the effect of fluoride on glycolysis is low in the tissues with low glycolysis [Dickens and Šimer, 1929] and also because the unaffected R.Q. is itself low.

In the experiment with rat testis addition of fluoride to glucose-Ringer quite definitely caused a lowering of carbohydrate metabolism. The extent of respiration, Q_{O_2}, is correspondingly reduced in the presence of fluoride in such extended experiments with tissues where a large part of the normal respiration is due to carbohydrate. The quotient in presence of lactate is quite unaffected by fluoride (*cf.* Table XII). This significant fact will be discussed later.

Insulin and respiratory quotient. The method used has much potential value in relation to the difficult question of the rôle of insulin in carbohydrate oxidation. The immediate question arising in the present paper was the effect of insulin on the carbohydrate metabolism in tumours, the insulin content of which has been shown to be less than that of most normal body tissues [Cramer, Dickens and Dodds, 1926]. In this connection the effect of adding insulin to the phosphate-Ringer solution was first studied for liver. Two experiments were made and the results are given in Table XIV. The insulin used had 14 international units per mg.

Table XIV. *Effect of insulin on* R.Q.

	Experiment no.	Concentration of insulin per cc. g.	R.Q. Without insulin	R.Q. With insulin
Liver	1	$1{\cdot}8 \times 10^{-5}$	0·86 0·87	0·92 0·95
	2	$1{\cdot}8 \times 10^{-5}$	0·83 0·81	0·83 0·77
Jensen rat sarcoma ...	1	7×10^{-7}	0·78 0·76	0·79 0·77
Rous chicken sarcoma	1	2×10^{-5}	0·94 0·95	0·95 0·91

In the first of these experiments a definite increase of carbohydrate metabolism was observed, the change being quite outside the limits of experimental error. In the second experiment, however, the same concentration of insulin had no effect whatsoever. It is evident that many further experiments will be necessary to account for this difference, and such questions as the state of nutrition of the animal, the particular lobe of liver used, etc. must be taken into account. In the two examples of tumours examined the results (Table XIV) indicate that the mere addition of insulin to tumours is insufficient to restore the property of oxidising carbohydrate in such tissues.

A note on the use of "oxantin" by tissues.

In an earlier paper [Dickens and Šimer, 1929] it was found that appreciable amounts of lactic acid were formed by normal and tumour tissue from "oxan-

tin" (dihydroxyacetone, Hoechst). On using a freshly purchased specimen of the same preparation, different values for the anaerobic glycolysis of tissues in dihydroxyacetone-Ringer solution were obtained. The purity of the preparation had previously been assumed to be sufficiently high, on the basis of the good yield of the dibenzoate (M.P. 121°) obtained by the method of Fischer and Taube [1924]. The variable results mentioned above caused us to purify the substance by distillation in high vacuum [Fischer and Mildbrand, 1924] of the portion of oxantin insoluble in a small quantity of acetone or alcohol. The distilled dihydroxyacetone (M.P. 67°–71·5°; M.W. 86) was only very slightly attacked by the tissues examined as the following examples show (Table XV). These values are of a similar order of magnitude to those obtained by Meyerhof and Lohmann [1926] with liver and kidney tissue. In all cases the readings fell somewhat during the experiments.

Table XV.

	Dihydroxyacetone			Dihydroxyacetone (90 %)+methylglyoxal (10 %)	
	0·1 %	0·2 %	0·5 %	0·1 %	0·3 %
Rat testis $Q_{CO_2}^{N_2}$	+2·5	+4·4	+4·6	+7	+20
Jensen sarcoma $Q_{CO_2}^{N_2}$	—	+2·5	—	—	—

That the impurity was methylglyoxal was shown by fractional distillation of the alcohol-soluble portion of oxantin *in vacuo*. The volatile distillate, when treated with hydrazine hydrate, gave a yield of nearly pure dihydrazone which amounted to 10 % by weight of the specimen of oxantin used, and after one recrystallisation from alcohol melted at 93° [Fischer and Taube, 1924]. The higher results obtained previously must therefore be ascribed to the great readiness with which tissue glyoxylases attack this impurity; the pure substance, as obtained by distillation of oxantin, must be used in all experiments with surviving tissues.

Discussion.

Significance of the measurements. The most obvious fact observed in these experiments is that all values for the R.Q. are within the limits of values corresponding to the oxidation of typical foodstuffs. This applies to the whole of the measurements on all the tissues, in some hundred experiments made up to the present. There is therefore no reason to suppose that the substances oxidised in isolated tissues under the conditions of our experiments are essentially different from those metabolised in the intact organism. Whilst the quotients for fat and carbohydrate will not differ appreciably in our experiments from those in the whole body, the same is not necessarily true of protein, the quotient for which is normally calculated after making allowance for the nitrogen excreted in the urine and fæces. The main nitrogenous waste product in the whole body is urea, but there is little evidence as to the extent of the urea formation by isolated tissues. The literature on this point has been discussed by Holmes and Watchorn [1927]. From Warburg's figures [1926] it is clear that at least a part of the nitrogen catabolised appears as ammonia, but

the formation of urea in surviving tissues cannot be excluded at present. The respiratory quotient for protein, if the end product is urea, is about 0·8; on the other hand it closely approaches the carbohydrate level if the end product is ammonia. This circumstance causes some uncertainty in the quantitative interpretation of our experimental figures. Fortunately the protein catabolism is not large in solutions containing glucose, if the figures for ammonia formation given by Warburg are taken as indicative of the extent of oxidation of proteins.

Relation between carbohydrate oxygen consumption and R.Q. If the protein metabolism may be neglected, an approximate idea of the percentage of the total respired oxygen used for the oxidation of carbohydrate can be gained from the relationship:

$$\% \text{ of total } O_2 \text{ for carbohydrate} = \frac{R - 0{\cdot}71}{1 - 0{\cdot}71} \times 100,$$

where R represents the observed value of R.Q. and the value 0·71 is taken as an average value of the R.Q. for the oxidation of fat. In the present state of knowledge of the metabolism of isolated tissues, it is necessary to consider for each individual case how far the application of such considerations is justifiable.

Liver. The mean value 0·79 obtained for rat liver would correspond to utilisation of only some 20–30 % of the total oxygen for combustion of carbohydrate in spite of the fact that about 0·2 % of glucose is present in the surrounding medium. There is no evidence from our experiments for the occurrence of any extensive transformation of fat into carbohydrate. Unless the carbohydrate formed is immediately oxidised, this process would result in a value of R.Q. much below the figures obtained.

Kidney. Kidney is one of the few tissues other than muscle and nerve upon which systematic measurements of R.Q. *in vitro* have been made. Shorr, Loebel and Richardson [1930] give several results, and average values of 0·88 and 0·85 in 0·2 % glucose as the means of two series of observations. Our values are in good agreement with these. If the protein oxidation is neglected, our mean quotient of 0·85 indicates that about 50 % of the respired oxygen is utilised for the oxidation of carbohydrate, a proportion considerably higher than in liver. In this particular case, however, we are probably not justified in neglecting the protein catabolism. Warburg [1926] states that in kidney the temperature coefficient showed that the principal source of the rather large excretion of ammonia was merely the washing out of ammonia from the tissue. If a more detailed investigation of the nitrogen metabolism of adult kidney were to show that this was at least in part a true ammonia formation from protein, the amount of oxygen required for protein would reduce the above figure for carbohydrate very considerably.

Testis. Schorr, Loebel and Richardson [1930] give one value of 0·97 for R.Q. of rat testis in Ringer-phosphate solution with glucose, but the value applies to a phlorizinised rat. The earlier paper of Loebel and Hickling [1927]

gives no figure for the value of R.Q. in the normal animal, although the effect of lactate upon R.Q. of rat testis is there briefly discussed. Our mean value of 0·94 for testis indicates a high carbohydrate metabolism, since probably about 80 % of the respired oxygen goes to the combustion of carbohydrate on the basis of this figure.

Brain. Our finding of a purely carbohydrate catabolism in brain is in good agreement with the recent results of Himwich and Nahum [1929] by the perfusion method. Loebel [1925] has found that rat brain in fructose has R.Q. = 0·99 and in lactate, 0·92. In Ringer solution without addition of nutritive substance the R.Q. was 0·86. Loebel gives no figures for the R.Q. in presence of glucose.

Retina. Retina occupies a quite exceptional position among all animal tissues, since it has the highest respiration and highest anaerobic glycolysis of any tissue yet studied [Warburg, 1927]. The aerobic glycolysis *in vitro* is also very high, and is considerably greater than in the case of most tumours. Our measurements show that, like all the other normal tissues with a high glycolysis, retina has a carbohydrate quotient.

Embryo. Since brain and retina are examples of nervous tissue, the high carbohydrate metabolism observed might be regarded as a special property of such tissues. It was therefore very desirable to extend our series of highly glycolysing normal tissues to include other examples, of which the most important is the embryo. Apart from the intrinsic interest of this problem, the comparison of embryonic tissue and tumours is one of the most likely methods of throwing some light on the different processes involved in the rapid but regulated growth of embryonic normal cells and the equally rapid but disorderly growth of tumour cells. The embryonic tissues studied by Warburg [1926] from this point of view showed a very high production of lactic acid under anaerobic conditions, which was removed almost completely when the tissues were given an adequate supply of oxygen. The carbohydrate quotient found for embryo in our experiments (Tables IV and V) may be compared with the mean value of about 0·90 obtained by Bohr [1900] for the mammalian foetus (guinea-pig).

The results obtained with the chicken embryo in a medium containing glucose are quite unlike the original observations of Hasselbalch [1900] on the metabolism of the whole egg, in which the mean value of R.Q. = 0·677 was obtained. The cause of this difference would repay further investigation. As the mean of eight experiments on chickens fed with oats and water, Regnault and Reiset [1849] obtained the value 0·927. There is no doubt from our experiments that the metabolism of the young (5-day) embryo is a purely carbohydrate one when the embryo is given an adequate supply of glucose. The anaerobic glycolysis of the 5-day chicken embryo determined by the bicarbonate method [Warburg, 1926, p. 131] is high; the embryo forms about 7 % of its own weight of lactic acid per hour ($Q^{N_2}_{CO_2}$ = 18·2, embryo dry weight = 9·0 mg.), a figure comparable with the anaerobic lactic acid production in

many tumours. The R.Q. of unity found by us is therefore like that of the other highly glycolysing normal tissues.

Tumour tissues. The experiments on rat tumours and on Rous sarcoma do not call for any special comment. The R.Q. is in all cases definitely below the carbohydrate level. Certain of the mouse tumours are of special interest particularly the spontaneous carcinoma II. In this tumour the respiration is large compared with the anaerobic glycolysis, consequently the U-value [Warburg, 1927] is negative, whereas it was formerly considered to be positive in all malignant tumours.

The value of the R.Q. however, shows that a large proportion of the substance oxidised consists of non-carbohydrate material and that the nature of the oxidation is the same in this as in other tumours.

Summing up the results on animal tumours the following generalisations apply to them all.

(1) The anaerobic glycolysis is high.

(2) Lactic acid is also produced aerobically.

(3) The respiration is of the same order of magnitude as in normal tissues, but

(4) the R.Q. is in all cases definitely below the carbohydrate level.

Whilst (1) applies to the normal tissues having a purely carbohydrate metabolism, and (2) applies also to retina and to testis under the conditions of our experiments, (4) distinguishes the tumour group from all the examples of highly glycolysing normal tissues studied.

Human tumours. Only a variable proportion of human tumour tissue is made up of cancer cells, and much of the material usually consists of connective tissue. The values for glycolysis and respiration are low in these two experiments for this reason, and the figures may be compared with measurements given by Warburg [1927] on similar material. It is probable that the actual cancer cells have a high glycolysis in this case also, since the adventitious tissue itself usually has a very low metabolic exchange. The low value of R.Q. is therefore in complete accordance with the measurements on other tumours, and may be contrasted with the quotient of unity given by highly glycolysing normal tissues.

Tuberculous lymphatic gland. This specimen, as will be observed from the figures shows a positive excess of fermentation over respiration [U-value, Warburg, 1927] and a high aerobic glycolysis (Table XI). Unless one is prepared to accept the view that the gland consisted of dying tissue this example proves an exception to all suggestions yet made for differentiating cancer from other normal and pathological growths on the basis of metabolic measurements. The R.Q. of the gland is also low and is comparable with that of tumours. How far similar disturbances of the respiration are present in other types of pathological growth is under investigation. The above example shows however that damage to the carbohydrate metabolism may occur in pathological growths other than cancer tissues under certain circumstances. In this particular

example the damage to the metabolism is also reflected in the figures for respiration and glycolysis. In other cases the lowering of R.Q. affords evidence of damage to the respiration which is not shown by the usual measurements of respiration and glycolysis. The measurement of R.Q. is therefore a valuable aid in extending the interpretation of pathological growth in terms of metabolism.

Conclusions.

In Table XVI are given the mean values for the R.Q. of normal tissues found in our experiments. The table also includes the value of the anaerobic glycolysis $Q_{CO_2}^{N_2}$, *i.e.* the amount of lactic acid formed per mg. dry-weight of tissue per hour in nitrogen (*e.g.* $Q_{CO_2}^{N_2} = 25$, indicates a production of lactic acid equal to 10 % of the dry-weight per hour; other values are proportionate). Inspection of the figures shows the following facts.

(1) Animal tissues may be divided into two main groups. In the first group, which includes most of the resting tissues, the value of R.Q. is definitely below unity, indicating the oxidation of a considerable proportion of fat. The carbohydrate oxidised forms sometimes only a small part of the total substance consumed by these tissues.

(2) In the second group, which includes nervous tissues like brain and retina, and also embryonic tissues, the R.Q. is almost exactly equal to unity, showing that the oxidative metabolism is a purely carbohydrate one.

(3) In the tissues of Group I there is a low anaerobic glycolysis, whilst in the tissues forming Group II there is on the contrary a high anaerobic catabolism of carbohydrate, as shown by the values of $Q_{CO_2}^{N_2}$ recorded in the above table. On the whole, increase of $Q_{CO_2}^{N_2}$ follows the increase in the value of R.Q. in Group I.

(4) Hence in normal tissues a low value of R.Q. is always associated with a low ability to form lactic acid under anaerobic conditions. On the other hand, if the anaerobic glycolysis of normal tissues exceeds a certain limit, the R.Q. is a purely carbohydrate one. There is a gradual transition from one type

Table XVI. *R.Q. and anaerobic glycolysis of normal tissues.*

Table	Tissue	R.Q.	$Q_{CO_2}^{N_2}$
IV	Liver	0·79	3 (W.)
III	Kidney	0·85	3 (W.)
IV	Intestinal mucosa	0·85	4 (W.)
,,	Submaxillary	0·87	7
,,	Spleen	0·89	8 (M. & H.)
,,	Testis	0·94	8 (W.)
,,	Embryo (rat, 10 mg.)	1·04	8 (W.)
V	Embryo (chicken, 10 mg.)	1·00	18 (W.)
IV	Brain cortex	0·99	19 (W.)
,,	Chorion (embryo wt., 30 mg.)	1·02	32 (N.)
VI	Retina	1·00	88 (W.)

Of rat unless otherwise stated.
W. = Warburg [1927], N. = Negelein [1925], M. & H. = Murphy and Hawkins [1925].
The other figures in the table are the mean values of our experiments.

to the other, and as will be seen from the figures given in Table XVI a definite parallelism exists between oxidative and anaerobic metabolism of carbohydrate in all the normal tissues studied.

These facts suggest very strongly the view that the oxidative removal of lactic acid in these tissues is concerned mainly or solely with carbohydrate. In those tissues where the anaerobic glycolysis is high, the R.Q. of unity indicates that carbohydrate is being removed by the oxidative metabolism, and the formation of lactic acid aerobically is thus diminished. On the other hand in tissues with low anaerobic glycolysis only a small portion of the respiration is concerned with carbohydrate, but this amount is sufficient to abolish the low anaerobic formation of lactic acid when these tissues are placed in oxygen. From the work of Meyerhof it follows that, at any rate in the case of muscle, the whole of the lactic acid is not oxidised, but a portion is resynthesised to carbohydrate. The energy for this process however appears from our figures to be derived in the tissues studied from the oxidation of carbohydrate and not from the combustion of other foodstuffs. There is nothing incompatible with such an assumption if the results are carefully examined. However the calculation of the exact numerical values of what may be called the "Meyerhof quotient for carbohydrate" *i.e.*

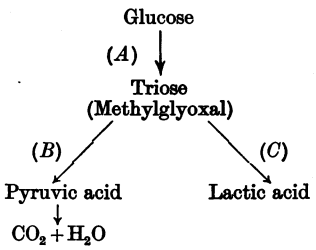
$$\frac{\text{Anaerobic glycolysis—aerobic glycolysis}}{\text{Respired oxygen used for combustion of carbohydrate}}$$

presents some uncertainties, arising not only from the difficulties discussed previously in the exact calculation of the proportion of oxygen used for carbohydrate from the R.Q. of excised tissues, but also from the special difficulties connected with the measurement of aerobic glycolysis. Owing to its indirect nature, the bicarbonate method of Warburg requires great care if reliable results for aerobic glycolysis are to be obtained, and a knowledge of the R.Q. of the tissue is presupposed in calculating the results. Apart from such technical considerations however, the effect of Ringer solution in some cases may cause definite damage to the efficiency of the Pasteur reaction. For example, rat testis forms an appreciable amount of lactic acid aerobically in Ringer solution, but not in serum [Warburg, 1926].

Whilst it is not yet possible, therefore, to state definitely if the relationship between the carbohydrate fraction of the respiration on the one hand and the glycolysis on the other takes the exact form of the Meyerhof quotient, it appears from our results that the removal of lactic acid, or the prevention of its appearance, is concerned with the oxidation of carbohydrate only.

This conclusion is supported by further experiments on the effect of lactate on the respiratory quotient of tissues (Table XII). Addition of lactate to excised tissues has been shown by Meyerhof and Lohmann [1926] to bring about an increase in the extent of respiration. Similar results were observed in the experiments on normal tissues summarised in Table XII, and in addition, measurement of the R.Q. showed a marked increase towards the carbohydrate level in both liver and testis. Clearly, lactate is utilised freely by these

tissues. In liver where the carbohydrate metabolism (aerobic and anaerobic) is normally low, the effect of lactate is even to raise the value of the R.Q. above the level in glucose. The figures for testis also show that glucose may largely be replaced by lactate in oxidation. It is difficult to express an opinion as to whether the lactate added is directly oxidised or is resynthesised into carbohydrate, an equivalent portion of which is then oxidised. Evidence from our experiments with fluoride sheds some light on this problem. Fluoride depresses the anaerobic breakdown of carbohydrate into lactic acid, and Table XIII shows that it also depresses the oxidative consumption of carbohydrate, as would be expected from the general considerations discussed below. On the other hand the second part of Table XIII shows that fluoride has absolutely no effect on the oxidative consumption of lactic acid. This result may best be considered with the aid of the simplified scheme given below, which is based on current views of intermediary carbohydrate catabolism. This scheme, which must be regarded as being made up of a series of more or less labile equilibria, is the one most in accordance with the facts contained in this paper:



In this system, fluoride inhibits carbohydrate catabolism somewhere along the line (*A*) while so far as is known it is without effect on (*B*) and (*C*). Consequently if it is assumed that the resynthesis of lactate into carbohydrate proceeds through methylglyoxal, the question as to whether the lactic acid itself, or an equivalent amount of carbohydrate is oxidised, is of a somewhat academic nature.

A more important question is the effect of methylglyoxal itself on tissue oxidation. The practical difficulty of the very rapid transformation of methylglyoxal into lactic acid by tissue glyoxalase has prevented us from studying this effect directly. An investigation of the influence on respiration of methylglyoxal in the presence of antiglyoxalase is much needed, and will be the subject of further experiments.

Effect of pyruvate. That pyruvate can support respiration of brain tissue after the latter has exhausted its own store of carbohydrate has been shown by Loebel [1925]. In the experiments with pyruvate recorded in Table XII the rise in the value of the R.Q. provides direct evidence that tissues possess the property of attacking pyruvic acid, although here also it is at present impossible to say whether this effect is preceded by a resynthesis to carbohydrate.

Tumours.

It has already been mentioned that according to current views the damage to respiration in tumours may be of two kinds: (1) the extent of respiration is diminished, and (2) the respiration loses its normal power of preventing glycolysis when the tissue is given an adequate supply of oxygen. Since the evidence concerning normal tissues brought forward in the present paper shows that the influence of respiration on glycolysis is not an effect of the respiration as a whole but is due to that part of the respiration which is concerned with the oxidation of carbohydrate, the metabolic deviation in tumours becomes much clearer than previously. The damage affecting oxidation processes in tumours is principally a damage of the oxidation of carbohydrate: tumours cannot oxidise carbohydrate to the same extent as normal cells having similar powers of anaerobic glycolysis. Experimental proof of the correctness of the statement is given in Table XVII in which the values for R.Q. of tumours determined in the experimental portion are compared with the values for anaerobic glycolysis.

Table XVII. *R.Q. and anaerobic glycolysis of tumour tissues.*

Table	Animal	Tissue	R.Q.	$Q^{N_2}_{CO_2}$
VII	Rat	Jensen sarcoma	0·82	34*
IX	,,	Slow sarcoma	0·94	18
,,	Chicken	Rous sarcoma	0·93	30*
IX & X	Mouse	Spindle-cell tar tumour 173	0·91	21
,,	,,	Tar carcinoma 2146	0·87	22
,,	,,	Crocker sarcoma	0·89	22
,,	,,	Sarcoma 37 S	0·86	27
,,	,,	Spontaneous tumour I	0·91	20
,,	,,	Spontaneous tumour II	0·87	(16)†
,,	Human	Papillary carcinoma of bladder	0·86	(3·4)†
,,	,,	Carcinoma of breast	0·84	(7·1)†

* [Warburg, 1926.] † Mixed tumours; much connective tissue.

If the figures given in this table are compared with those given above (Table XVI) for normal tissues having similar powers of glycolysis ($Q^{N_2}_{CO_2}$), the damage to the oxidation of carbohydrate in tumours is evident from the fact that whereas all such normal tissues have a purely carbohydrate R.Q., in the case of tumours the R.Q. is in all cases below unity, and in many cases indicates the probable consumption of large amounts of non-carbohydrate material. The tumour cell like other rapidly growing cells has a high glycolysis under anaerobic conditions. Since the lactic acid arises from the glucose originally contained in the surrounding medium, this must mean that the tumour cell is capable of producing freely the intermediary compounds in the chain of processes the overall result of which is the transformation of glucose into lactic acid. Referring to the scheme of intermediate metabolism given above, in tumours and highly glycolysing normal tissues the reactions along paths (*A*) and (*C*) occur very rapidly under anaerobic conditions resulting in a liberal production of lactic acid. Since all tissues attack methylglyoxal with very great rapidity, converting it into lactic acid, there is no reason to suppose that

this stage (*C*) is ever defective in normal or tumour tissue. Path (*A*), *i.e.* the conversion of hexose to the 3-carbon system, is therefore the stage which must control glycolysis in those tissues where the glycolytic power is low (*e.g.* liver and kidney).

If this principle is accepted, since only minimal amounts of the intermediate 3-carbon system will be available for oxidation, a simple explanation is available as to why these tissues also utilise so little carbohydrate in their oxidative catabolism. The deficiency in normal tissues with low glycolytic power is in the primary stages of the breakdown of hexose, thus affecting both aerobic and anaerobic catabolism of carbohydrate. In normal tissues with high glycolysis, the corresponding carbohydrate R.Q. shows that there is no deficiency in stage *B*, and that the oxidation of the intermediate compound, once this is formed, proceeds quite readily in normal tissues.

In tumours the conditions regulating the carbohydrate metabolism are entirely different. Stages *A* and *C* proceed quite as freely as in normal highly glycolysing tissues; there is thus a liberal production of the intermediate 3-carbon compound, yet the oxidation of carbohydrate is defective, as is shown by the fact that the value of the R.Q. is definitely below unity in all cases. The simplest and most probable explanation of these circumstances is that the failure of oxidation of carbohydrate in tumours occurs at a stage following the formation of the methylglyoxal. Such damage would have no effect on the anaerobic glycolysis, but would reduce the amount of carbohydrate capable of being removed by oxidation when the tumour tissue is transferred to aerobic conditions. In tumours there is therefore inadequate oxidation of carbohydrate, as a result of which is found a low R.Q. and the occurrence of aerobic glycolysis.

Since several stages are undoubtedly concerned in the oxidation of the 3-carbon intermediate, finally resulting in the formation of CO_2 and water, it is of importance to investigate this chain of reactions (route *B*) more closely. There is considerable evidence that one of the most important products is pyruvic acid. This substance would result from the simple dehydrogenation of either the hydrate of methylglyoxal, or, rather less probably, of lactic acid itself. The rise in R.Q. accompanying the addition of pyruvate to all tissues studied including tumours (Table XII) shows clearly that tumour tissue retains the power possessed by normal tissue of attacking pyruvate. On the other hand addition of lactate does not appreciably influence the oxidative carbohydrate metabolism in tumours, which in this respect show a behaviour in sharp contrast to that of normal tissues with a similar respiratory quotient, such as liver and kidney. The damage to the carbohydrate metabolism in tumours therefore probably precedes the formation of pyruvic acid in the chain of intermediates, and is subsequent to the transformation of hexose to the earlier members of the 3-carbon stage.

To what extent, qualitatively or quantitatively, this metabolic deficiency is limited to tumours cannot be discussed until much more evidence has been

collected on the value of R.Q. It is however clear from the measurements on the tuberculous lymphatic gland recorded in Table XI that definitely non-neoplastic types of growth may show damage to respiration which, as far as our observations show, is similar to that in cancer tissue. In this connection Warburg's [1929] classification may be referred to. In Warburg's view, the occurrence of aerobic glycolysis is not specific for tumours, unless one considers as an additional criterion the power of the tissue in turning to account the energy resulting from glycolysis. Tumour tissue is characterised by its power of growth, and a part of the energy needed for growth arises from such glycolysis. On the other hand tissues other than tumours possessing the power of aerobic glycolysis are considered by Warburg to be made up of dying cells. There are several difficulties in accepting this view. The metabolic distinction between malignant growth and the benign tumours, the powerful aerobic glycolysis of which was observed by Warburg [1926], is not at all marked, although clinically and morphologically the distinction is, of course, fundamental. The result obtained with the mammalian retina, which all measurements yet made have shown to possess a very high aerobic glycolysis, is attributed by Warburg firstly to the special function of the retina, and secondly to damage occurring on removing this organ from the body. Apart from these anomalies there are difficulties in interpreting the results in any particular instance, particularly in deciding whether the tissue consists of dying cells, as in the case of the tuberculous gland described above.

The very important discoveries of Warburg and his collaborators may be briefly summed up, as far as they concern tumours, by the statements that in tumours the respiration is injured, and that as a result of such injury glycolysis appears aerobically [Warburg, 1929]. The experiments reported in the present paper show that the damage to respiration in tumours is wholly or mainly a damage to the oxidation of carbohydrate. The whole question as to whether such injury is in any way specific for tumours, and in general whether the difference between tumours and other types of pathological growth is capable of being adequately expressed in terms of metabolism, must be left to future discoveries to prove or disprove.

SUMMARY.

1. The method for measurement of R.Q. previously described (Part I) has been applied to a series of normal and tumour tissues.

2. The validity and accuracy of the method are shown (*a*) by the regularity of respiration over long periods, and (*b*) by the agreement between duplicate determinations of R.Q.

3. All results indicate the consumption of normal foodstuffs in respiration, without appreciable interconversion of one form into another.

4. Only certain tissues have a carbohydrate quotient; these are the highly glycolysing normal tissues, and include brain, retina, chorion and embryo.

5. Normal tissues with low glycolytic power consume largely non-carbohydrate material, presumably fat (liver, kidney, mucous membrane).

6. A group intermediate both in glycolysis and value of R.Q. comprises submaxillary, spleen and testis.

7. All the tumours examined had R.Q. definitely below the carbohydrate level, in spite of their very marked ability to convert glucose into lactic acid.

8. The oxidative metabolism of tumour is thus quite distinct from that of normal tissues, since tumours possess both high glycolysis and low oxidative carbohydrate metabolism, a combination not found in normal tissues.

9. It is concluded that there is a definite defect in the oxidation of carbohydrate by tumour tissue.

10. Consideration of the actual values of R.Q. obtained, supports the view that in all the tissues examined the energy for resynthesis in the Meyerhof cycle, is provided by oxidation of carbohydrate rather than of other foodstuffs.

11. Tumour tissue does not utilise lactic acid added to the medium and differs in this respect from normal tissues having a similar value of R.Q.

12. Tumour tissues and normal tissues can utilise pyruvic acid freely in their oxidative metabolism.

13. Evidence has been obtained that fluoride inhibits oxidative as well as glycolytic decomposition of carbohydrate. Fluoride does not affect the R.Q. or respiration of normal tissues (rat testis) in the presence of an adequate supply of lactate.

14. The effect of the addition of insulin to the medium is described.

15. The general conclusion from the evidence summarised above is that the failure of the oxidation of carbohydrate by tumour tissue occurs at a stage of the intermediary metabolism subsequent to the conversion of hexose to the 3-carbon stage, and prior to the oxidation of the latter.

16. The probable nature of the different types of carbohydrate metabolism occurring in normal tissue and tumours is considered on the basis of the above hypothesis. The bearing of these observations on the more general question of the possibility of the interpretation of the behaviour of tumours by means of the metabolic measurements is discussed.

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