CVIII. THE METABOLISM OF NORMAL AND TUMOUR TISSUE.

IV. THE RESPIRATORY QUOTIENT IN BICARBONATE-MEDIA.

By FRANK DICKENS and FRANTIŠEK ŠIMER.

From the Courtauld Institute of Biochemistry, Middlesex Hospital, London, W. 1.

(*Received June 30th, 1931.*)

The experiments described in Part II of this series [Dickens and Šimer, 1930] demonstrated the existence of a definite relationship between the aerobic and anaerobic metabolism of carbohydrate by normal tissues. As has often been pointed out, the figures for total oxygen consumed in respiration and those for anaerobic glycolysis appear to bear no mutual relationship whatsoever [*e.g.* Meyerhof, 1930, p. 178]. When, however, the previous observations were completed by the addition of measurements of the R.Q. and, instead of considering the relationship of the total respiration to the glycolysis, we compared the glycolysis with that fraction of the respiration corresponding to the oxidation of carbohydrate, it was then found that these two sets of values ran parallel in all the types of normal tissue studied. In tumours, on the other hand, there is a definite damage to the oxidation of carbohydrate, so that the amount of carbohydrate oxidised to CO_2 and water is smaller than would correspond with the high value of anaerobic glycolysis in such tissues.

In the above experiments, the tissue was suspended for the purpose of the measurements in a Ringer solution buffered with phosphate. It was considered desirable to ascertain if this relationship is generally applicable, and particularly whether it holds in the case when bicarbonate-media such as serum or bicarbonate-Ringer, are used. For this purpose it was necessary to evolve a method suitable for the measurement of R.Q. under these conditions. The present paper concerns the application of this method to the above problem and the comparison of the R.Q. and respiration of tissues in bicarbonate- and phosphate-media.

It may be stated at once that the conclusions of Part II hold good under the new conditions and are therefore independent of the particular medium used for the experiments. The detailed discussion of the results is left until after the description of the experiments.

Experimental.

The method used for the determination of R.Q., respiration and aerobic glycolysis has been fully described in Part III [Dickens and Šimer, 1931]. The tissue, cut into thin slices (except when, as with the retina or embryo, the intact material is suitable for use without cutting), is suspended in bicarbonate-Ringer solution [Warburg, 1930] or horse-serum, the latter being previously inactivated by heating to 56° for 1 hour. In all experiments glucose (0·2 %) was added to the medium before use. The serum used contained a normal amount of lactic acid (25–30 mg./100 cc.).

It is necessary to repeat here that, for the sake of accuracy in the R.Q., the total oxygen consumption must not fall much below 300 mm.^{3} and, on the other hand, the establishment of diffusion equilibrium is endangered, particularly in experiments in serum, by a more rapid gas exchange than about 100 mm.^{3} per hour. Hence, in general, the experiments lasted for about 3–5 hours; the figures given for respiration and glycolysis are, therefore, the mean values over the whole of this period.

Accuracy of the method.

Table I contains the results of a number of duplicate experiments by the new method, made simultaneously on different slices of the same piece of tissue or, in the case of embryo, by the use of two embryos of the same age. The Table shows that the accuracy of the method is sufficient and is in agreement with that to be expected from the considerations given in the previous paper.

Table I.

Tissue	Serum or Ringer solution	R.Q.
Liver	S	0·72, 0·71
	S	0·73, 0·71
Kidney	R	0·81, 0·82
Testis	R	0·88, 0·87
	R	0·93, 0·92
	S	0·93, 0·92
Brain cortex	R	0·99, 0·99
Chicken embryo	R	0·99, 0·98
Jensen sarcoma	R	0·75, 0·73
	S	0·79, 0·79
Slow-growing sarcoma	S	0·77, 0·77

Results.

The results of all measurements are collected in Table II. The tissues selected are representatives of groups with differing degrees of anaerobic glycolysis. A typical protocol of an experiment, showing the details of an actual measurement and also the method of calculation, was given at the end of Part III.

R.Q. in bicarbonate and in phosphate. In a number of experiments simultaneous duplicate determinations of R.Q. were made on slices from the same

Table II. *Metabolism in bicarbonate and phosphate.*

Specimen no.	Tissue	Bicarbonate-medium: Ringer or serum	Bicarbonate-medium: $NaHCO_3$ m-mols	Bicarbonate-medium: CO_2 in gas mixture vols. %	Bicarbonate-medium: R.Q.	Bicarbonate-medium: Q_{O_2} (respiration)	Bicarbonate-medium: $Q_{CO_3}^{O_2}$ (glycolysis)	Phosphate-medium: R.Q. (Same specimen of tissue as in bicarbonate)	Phosphate-medium: Q_{O_2} (Same specimen of tissue as in bicarbonate)
	Liver								
6	Fasted	R	14	1·3	**0·55**	13·4	2·9	—	—
7	Fed	R	14	1·3	**0·86**	9·7	2·1	—	—
9	Fasted 12 hrs.	R	14	1·3	**0·77**	10·7	1·4	**0·71** **0·68**	7·6 6·4
22	Fasted	S	20	4·5	**0·72** **0·71**	9·1 8·5	0·9 0·9	**0·65** **0·63**	9·9 10·2
23	Fasted	S	20	4·5	**0·73** **0·71**	12·7 11·2	1·3 1·6	**0·58** **0·58**	8·6 9·5
35	Fed	S	20	4·5	**0·76** **0·73**	10·0 9·3	2·3 2·6	— —	— —
36	Fasted	R	20	4·5	**0·66** **0·64** **0·66**	13·7 13·9 13·5	4·2 4·3 4·1	**0·68** **0·68**	11·4 11·2
	Kidney								
1	—	R	14	3·2	**0·82**	18·9	0	—	—
2	—	R	14	1·9	**0·78**	19·1	0	—	—
18	—	R	25	4·5	**0·81** **0·82**	22·1 20·7	0 0	**0·85** **0·82**	20·7 18·0
19	—	S	20	4·5	**0·89**	22·8	−6·2	—	—
28	—	S	20	4·5	**0·86**	22·8	−3·2	**0·86** **0·87**	23·0 26·0
	Testis								
5	—	R	14	—	**0·87**	10·2	2·8	—	—
12	—	R	25	—	**0·88** **0·87**	8·5 7·3	4·3 4·6	**0·88** **0·87**	7·7 8·6
20	—	R	25	—	**0·93** **0·92**	10·0 10·5	4·9 5·5	**0·89** **0·91**	9·3 9·1
21	—	S	20	—	**0·93** **0·92**	8·4 9·1	2·8 2·6	**0·96** **0·93**	8·5 7·9
	Brain cortex								
3	—	R	14	1·3	**1·00**	10·6	1·6	—	—
4	—	R	14	1·3	**0·99**	12·8	1·2	—	—
11	—	R	25	5·0	**1·00**	16·2	0·3	—	—
15	Young rat	R	25	4·5	**0·99** **0·99**	14·0 14·8	2·5 2·1	— —	— —
16	Old rat	R	25	4·5	**0·96** **0·94**	15·2 14·4	3·2 2·7	— —	— —
17	Young rat	S	20	4·5	**1·04**	13·9	(3·9)	—	—
29	Senile rat	R	25	—	**0·95** **0·95**	10·0 10·0	2·7 3·2	— —	— —
	Chorion								
10	From embryo of dry wt. 145 mg.	R	25	4·8	**0·98**	25·4	7·4	—	—
25	From embryo of dry wt. 161 mg.	S	20	4·5	**1·02**	26·9	−0·9	—	—
	Retina								
31	—	R	20	4·5	**1·00**	19·5	26·9	—	—
32	—	R	20	4·5	**1·03**	18·8	19·7	—	—
	Embryo (at 40°. Chicken 4th day)								
33	(Dry wt. 4·10 mg.)	R	20	4·5	**0·98**	11·3	1·3	—	—
34	(Dry wt. 3·08 mg.)	R	20	4·5	**0·99**	11·6	3·2	—	—
	Jensen sarcoma								
13	—	R	25	4·5	**0·75** **0·73**	9·5 9·0	8·9 8·9	**0·80** **0·78**	8·5 8·0
26	—	R	25	4·5	**0·84** **0·77**	10·1 10·0	10·4 16·0	— —	— —
27	—	S	20	4·5	**0·79** **0·79**	8·0 8·8	9·6 11·6	**0·79** **0·79**	8·7 11·1
	Slow-growing sarcoma								
30	—	S	20	4·5	**0·77** **0·77**	5·6 4·3	9·8 7·0	**0·88** **0·90**	5·5 6·3

piece of tissue suspended (*a*) in bicarbonate-Ringer or serum, and (*b*) in phosphate. The compositions of the bicarbonate-Ringer and phosphate-Ringer solutions used were identical in respect to glucose (0·2 %), calcium and potassium, and the concentration of bicarbonate was also equal to that of secondary phosphate. Such control experiments with phosphate-Ringer were not made with those tissues previously shown to have a R.Q. of unity in this medium (brain, embryo, retina, chorion).

Reference to these individual experiments, which are included in Table II, shows that with the exception of liver tissue (see below) there is no systematic difference in the values of R.Q. observed in the two types of media, nor any significant difference in R.Q. due to the use of either serum or Ringer solution, or to the different CO_2 tensions or bicarbonate concentrations shown in the Table. These facts are also fully supported by a comparison of the mean values of R.Q. These figures are given in Table III, in which the mean values obtained in the numerous experiments in Part II are compared with the mean of the figures in bicarbonate-media obtained in the present paper.

Table III.

Tissue	Bicarbonate-medium. Mean R.Q.	Phosphate-medium R.Q. Mean of all previous experiments
Liver	0·71	0·79
Kidney	0·83	0·85
Testis	0·90	0·94
Brain cortex	0·98	0·99
Embryo (chicken)	0·99	1·00
Chorion (rat)	1·00	1·02
Retina	1·01	1·00
Jensen sarcoma	0·78	0·83
Slow-growing sarcoma	0·77	0·94

From Table III it is clear that if the tissues are arranged according to the mean value of R.Q., not only the order of the various tissues in the series is the same, but in addition to this, in the case of each individual tissue, the values of R.Q. in phosphate and bicarbonate respectively differ only slightly. In all normal tissues the difference does not exceed the variation between the measurements on different specimens of the same tissue measured in the same medium. However, it may be significant that in all cases the mean R.Q. in bicarbonate is very slightly below that in phosphate.

In the two types of tumour examined this difference, though in the same direction, is more pronounced. The number of specimens examined as yet is insufficient for any general conclusion to be drawn from this fact, but it is sufficient to note that in all examples the R.Q. isdefinitely below unity, and is in fact lower in bicarbonate or serum than in phosphate.

R.Q. of liver tissue. In a number of experiments in phosphate-media values of R.Q. below the fat-level have been obtained with livers of fasted rats (Table IV). The time since last giving food was in most cases 24 hours. Water was present in the cages throughout. These low quotients are not observed

Table IV. *R.Q. of liver of fasted rats measured in phosphate-Ringer solution, 0·2 % glucose, 38°.*

R.Q.	Q_{O_2}
0·61	8·9
0·57	7·1
0·59	7·8
0·62	8·4
0·62	8·7
0·65	9·9
0·63	10·2
0·58	8·6
0·58	9·5

in the case of animals which have been fed during a period of about 12 hours preceding the experiment. Consequently, they were not encountered in the series of rats used for the experiments in Part II, since in these experiments all the animals were fed either on the day of the experiment or on the evening of the previous day.

When similar experiments with fasted and with fed animals were made in bicarbonate-Ringer solution and in serum, it was found that the measurements in Ringer solution resembled those in the phosphate-medium, in that values of R.Q. below the physiological level were obtained with fasting animals. In serum, on the other hand, the value of R.Q. was within the normal limits and corresponded with the value found for the oxidation of fats.

R.Q. of brain tissue. In some of the experiments with cerebral cortex of rats, quotients a little below unity were obtained (Table II). It was noted that in all these experiments old rats had been used; in one example (specimen 29), R.Q. = 0·95, a very old animal aged about 2 years was used. The results suggest a tendency for the R.Q. to fall a little below the carbohydrate level with advancing age of the rat in the case of brain tissue.

Measurements of respiration (Q_{O_2}) *by the new method.* Reference to the figures in Table V shows that the extent of respiration as measured by the direct method used in this paper is in agreement with that found by Warburg [1930] by the use of his bicarbonate method. In view of the fact that our

Table V.

Tissue	Q_{O_2} This paper (mean value)	Q_{O_2} Warburg [1927]
Kidney	21·1	21
Liver	11·3	12
Testis	8·4	12
Brain	13·2	11
Chorion	26·2[1]	14·4[2]
Retina	19·2	17[3], 31[4]
Embryo	11·5[5]	10[5]
Jensen sarcoma	9·2	9

[1] Embryo dry weight 150 mg.
[2] Embryo dry weight 15·4 mg. [Negelein, 1925].
[3] Warburg, Negelein and Posener [1924].
[4] Warburg, Posener and Negelein [1924].
[5] Chicken embryo, weights 3·5 and 1·7 mg. respectively (dried at 110°).

measurements extend over a period of 3–5 hours, and are therefore not maximal but mean values over this period, this agreement is quite satisfactory, and shows that in most cases the respiration remained nearly constant over this long period. In one instance, that of chorion, the respiration observed on two separate specimens was found to be considerably higher than that given by Negelein [1925]. The latter, however, refers to the membrane obtained from smaller embryos than those which provided the material for our experiments.

Measurements of glycolysis ($Q^{O_2}_{CO_2}$). The new method has the advantage over the phosphate method that it permits the simultaneous measurement of aerobic glycolysis, as well as respiration and R.Q., on the same piece of tissue. The method is the only one suitable for the detection of slight aerobic formation of acid, either in Ringer solution or serum. From Table II it will be seen that, under the conditions of these experiments, nearly all normal tissues produce small amounts of acid, and this is true whether the medium for the experiment be serum or bicarbonate-Ringer. It will be noticed, for instance, that in the experiment with testis the acid production diminishes but does not disappear completely even in serum.

The only exceptions to the above statement in the series of tissues studied, were kidney and chorion. With kidney in Ringer solution the bicarbonate content in all four experiments was, within the limits of experimental error, quite unchanged during the whole period of the experiment. In serum, on the other hand, kidney caused a definite increase in the content of bound carbon dioxide over and above that corresponding to the retention of respiratory carbon dioxide by the serum (specimens 19 and 28, Table II). In the case of chorion, the appreciable aerobic glycolysis found in Ringer solution not only disappears in serum [Negelein, 1925] but there is even a slight consumption of fixed acid in the latter medium (specimen 25).

It should be remarked that knowledge of the nature of the traces of acid produced aerobically is at present inadequate. Owing to the small amount of acid formed in such cases it is very difficult to control the manometric observations on this point by reliable chemical methods of analysis, and it cannot yet be definitely assumed that all the acid formed is lactic acid in the case of the feebly glycolysing normal tissues.

A further complication in interpreting these data for the aerobic glycolysis of normal tissues, is the possibility of a portion of the acid formed being neutralised by a simultaneous ammonia-liberation by the tissue. For instance, in the case of kidney even in the presence of glucose an amount of ammonia (0·9 mm.^{3} per mg. tissue per hour [Warburg, 1930]) comparable with the figures for aerobic glycolysis found in the other examples of normal tissues in Table II (*e.g.* in liver tissue), appears in the solution in which the kidney tissue is suspended.

Turning to the highly glycolysing tissues such as the retina and tumours, it will be seen from Table II that the figures for aerobic glycolysis are lower

than those given by Warburg's bicarbonate method. This is due to the extended period of the experiments and the consequent appreciable lowering of the bicarbonate content of the Ringer solution or serum.

It is not possible in such cases to combine maximum accuracy in determining R.Q. with determination of glycolysis under optimal conditions. In highly glycolysing tissues separate experiments of short duration, such as those of Warburg [1930], must be used for obtaining a standard value of $Q_{CO_2}^{O_2}$.

Discussion.

Effect of medium on respiration and R.Q.

Phosphate- and bicarbonate-Ringer solutions. As was described in Part II, the extent of the respiration of surviving tissues does not suffer when the bicarbonate buffer in the medium is replaced by phosphate. This fact has also been well recognised in work on isolated muscle [Meyerhof, 1930, p. 53], and it is only in the case of other tissues that the suitability of phosphate appears to be questioned. The experiments described in the present paper show that the R.Q. is also very nearly the same in bicarbonate-media as in the simple phosphate-buffered Ringer solution previously used. Hence the respiration is both quantitatively and also, at least as far as concerns the type of metabolites oxidised, qualitatively independent of the nature of the buffer solution in which the tissue is suspended. As far as the available evidence shows, the state of the tissue in these different media seems to differ only in respect of its behaviour under certain quite special conditions. A much discussed example of this is provided by the action of cyanide in the two media [Dixon and Elliot, 1929; Alt, 1930; Warburg, 1931].

Serum and Ringer solution. The evidence previously advanced in favour of the use of serum as a medium for measurement of tissue metabolism was the effect on the aerobic glycolysis [Warburg, 1930]. In our experiments there is in most cases a tendency for aerobic glycolysis to be diminished in serum (*e.g.* in the case of testis), a fact which may be interpreted as indicating some damage to the tissue in the simple salt solution. The best example is given by chorion in which the appreciable aerobic glycolysis observed in Ringer-bicarbonate disappears when the tissue is suspended in serum (specimen 25). A new instance observed in the present paper is the effect of serum on the abnormally low R.Q. observed with the livers of starved animals in simple salt solutions. Here the damage to respiration in Ringer solution is shown by a value of R.Q. which may be compared with that of the atypical type of oxidation found in mechanically damaged tissue and in tissue extracts. That the effect of serum in this case is not to be explained by consumption of lactic acid contained in normal physiological concentration in this medium is shown by the fact that in all three experiments in serum (specimens 22, 23, 35), there was no disappearance of fixed acid from the medium (see, for instance, protocol at end of Part III) either with fasted or with fed animals. The con-

centration of lactate in the serum, however, was sufficient to cause a marked consumption of this substance by kidney tissue in serum (specimens 19 and 28).

It is proposed to investigate more fully this question of the consumption of lactic and pyruvic acids by tissues, for which purpose the method used in this paper is particularly suitable. One of the problems awaiting solution is the discrepancy between the above findings and the utilisation of lactic acid by liver *in vivo*. *In vitro*, in the presence of a large concentration of lactate, there is a definite increase in the respiration with accompanying consumption of lactate [Meyerhof and Lohmann, 1926] and increase of the value of R.Q. [Dickens and Šimer, 1930] of liver tissue above the level in glucose. Meyerhof and Lohmann [1926] also observe an action of serum in increasing the respiration of liver tissue which they attribute to the same cause, namely the presence of lactate in the serum. Our experiments on this point do not support this view, since no disappearance of lactic acid was observed, at least from serum of normal lactic acid content and containing an adequate supply of glucose. The same authors give some low figures for R.Q. of the liver of a starved rat in serum. The serum used by them however was acidified and the CO_2 removed by evacuation.

Relationship between respiration and glycolysis. The values in Table III and the agreement of individual comparisons in phosphate and bicarbonate (Table II) are sufficient to establish the correctness of the values of R.Q. previously measured. All quotients not only in normal tissues, but also in tumours, lie between the limits corresponding to the complete oxidation of normal foodstuffs.

The regular relationship previously found between the oxidative and anaerobic metabolism of carbohydrate in normal tissues is confirmed by measurements made under physiological tension of CO_2 in serum or bicarbonate-Ringer solution. Under the same conditions the R.Q. of tumour tissue is definitely below the carbohydrate level, and indicates a damaged oxidation of carbohydrate by such tissue.

The relationship found for other normal tissues can also be extended to the case of resting muscle. The value of R.Q. is below the carbohydrate level in this tissue (*e.g.* for diaphragm of the mouse R.Q. = 0·84, mean of six experiments in phosphate Ringer with glucose), a fact which is in accordance with the value of anaerobic glycolysis ($Q^{N_2}_{CO_2} = 3$–4). Unfortunately, similar measurements on working mammalian muscle are not available. A more general illustration of this relationship is provided by the developing embryo. As was shown by Negelein [1925] the glycolytic power of embryonic tissues falls regularly during development. Our measurement of R.Q. of young (4–5 day) chicken embryos shows that the oxidative metabolism of such embryos is a purely carbohydrate one. Needham [1931] in his exhaustive discussion of the probable nature of foodstuffs utilised during embryonic development, regards the development of the chick embryo as being divided into three periods of which the first, corresponding to the first five or six days

of incubation, is a period of carbohydrate oxidation; the second a period of protein utilisation and in the last stage the energy is derived mainly from the oxidation of fat. Corresponding with the disappearance of free glucose in the first eight days, an accumulation of lactic acid was found by Tomita [quoted by Needham, 1931]; this subsequently falls to a low level. All these facts are in complete agreement with our general conclusion that the replacement of an oxidative metabolism of carbohydrate by one of fat is associated with a corresponding lowering of the glycolytic power; the oxidative and glycolytic cleavage of carbohydrate proceeding hand in hand in this as in other cases.

SUMMARY.

1. The method for the measurement of R.Q., respiration and aerobic glycolysis previously described (Part III) has been applied to a series of normal and tumour tissues.

2. The accuracy of the method is shown by the good agreement between duplicate determinations.

3. No significant difference has been observed in the values of R.Q. and respiration measured in salt solutions buffered (*a*) by bicarbonate under physiological tension of CO_2, or (*b*) by phosphate in a CO_2-free atmosphere.

4. The respiratory quotients of the tissues of fed animals are in all cases within the limits corresponding to the oxidation of normal foodstuffs.

5. In fasting animals, the R.Q. of liver tissue in simple salt solutions containing glucose falls below the physiological level. It is restored to the fat level when the measurement is made in serum.

6. The conclusions derived from the experiments in phosphate-media previously reported (Part II) are established for bicarbonate-media also, and some new examples of the relationship between oxidation of carbohydrate and its glycolytic cleavage are brought forward.

We wish to thank Prof. S. Russ and Prof. J. McIntosh for tumour material used in these experiments.

One of us (F. D.) is working as a member of the scientific staff of the Medical Research Council and the other (F. Š.) is in receipt of a scholarship from the Halley Stewart Trust Fund.

REFERENCES.

Alt (1930). *Biochem. Z.* **221**, 498.
Dickens and Šimer (1930). *Biochem. J.* **24**, 1301.
—— —— (1931). *Biochem. J.* **25**, 973.
Dixon and Elliot (1929). *Biochem. J.* **23**, 812.
Meyerhof (1930). Die chemischen Vorgänge im Muskel. (Springer, Berlin.)
—— and Lohmann (1926). *Biochem. Z.* **171**, 381.
Needham (1931). Chemical embryology (in press). (Univ. Press, Cambridge.)
Negelein (1925). *Biochem. Z.* **165**, 122.
Warburg (1927). *Biochem. Z.* **184**, 484.
—— (1930). Metabolism of tumours. (Constable, London.)
—— (1931). *Biochem. Z.* **231**, 493.
—— Negelein and Posener (1924). *Klin. Woch.* **3**, 1062.
—— Posener and Negelein (1924). *Biochem. Z.* **152**, 309.