THE RESPIRATION OF *HELIX POMATIA*, A BALANCE SHEET

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INTRODUCTION

When one isolates a tissue from a living animal and carries out a physiological experiment upon it, one often assumes that the results obtained will be applicable to the tissues in the intact animal. This has proved a useful working hypothesis though there are few investigations into the validity of the assumption (Barcroft, 1908; Field, Belding & Martin, 1939; Martin & Furhmann, 1941, 1955).

The gastropods, and especially the snails, provide very suitable material for such an investigation, since their tissues are bathed in a blood sinus and do not have a specialized high-pressure blood supply canalized to each organ. With this in mind we have investigated both the respiration of the isolated organs of the snail and the respiration of the intact animal to see whether the sum of the parts added up to the respiration of the whole animal.

METHOD

Starved *Helix pomatia* were kept in a refrigerated cold room at 2–5° C. They remained in an inactive state throughout the period of captivity though none of them developed a calcareous epiphragm. The respiration measurements were carried out on thin slices of tissue placed in Warburg manometers. Unless otherwise stated the gas phase was air. Both the direct and the indirect methods were used (Umbreit, Burris & Stauffer, 1945). The manometer bath was kept at $28 \pm 0{\cdot}01°$ C., this being the temperature at which Baldwin (1938) carried out his studies on the metabolism of the snail liver. After each experiment the tissue was removed from the manometer, dried overnight and weighed.

The respiration of the whole snail was measured by the direct method, a simple container being fitted to the standard Warburg manometer. The bath was kept at 28° C. and the snails lived quite well at this temperature provided that the humidity was kept high. This temperature was well within the thermal limits of the animals; Hogben & Kirk (1944) state that snails will live for several days at 35° C. under humid conditions, whilst their upper thermal death temperature is 44° C. During the course of long-term experiments on the respiration of the intact animals it was periodically necessary to replenish the air in the chamber. This was followed by a period of re-equilibration.

RESULTS

(1) *Respiration of isolated tissues*

One of the first problems was the choice of a physiological solution to bathe the isolated tissue. Previous workers such as Baldwin had used a phosphate solution containing in each litre, 16·12 g. $Na_2HPO_4.12H_2O$., 0·69 g. $NaH_2PO_4.H_2O$, 0·14 g. NaCl, 0·443 g. KCl. It will be seen that this solution is mainly a phosphate solution bearing little relation to the constituents of normal snail blood (Lustig, Ernst & Reuss, 1937), though Baldwin took care to ensure that it was isotonic with snail blood and had the same pH and Na/K ratios. Rees (1953), in his investigation into the enzyme systems present in the liver of the snail, suspended his material in either 0·5% KCl or 0·2 M-sucrose.

We decided to compare the respiratory activity of the tissues in Baldwin's solution and in a standard Ringer solution. For our Ringer solution we took Krebs–Ringer containing 0·15 M solutions of NaCl, KCl, KH_2PO_4, $MgSO_4.7H_2O$, and 0·11 M-$CaCl_2$ made up in the ratio of 100:2:1:1:3. It was buffered to pH 7·4 with phosphate buffer.

Table 1. *The respiratory rates of isolated tissues from* Helix pomatia *immersed in Baldwin's phosphate solution and Krebs'–Ringer*

Q_{O_2} = μl. oxygen/mg. dry weight/hr.

	Tissue	Readings	Q_{O_2} Baldwin	Q_{O_2} Krebs
1	Cerebral ganglia	20	4·00 ± 2·3	2·61 ± 1·7
2	Pedal ganglia	18	2·89 ± 1·6	2·53 ± 1·5
3	Liver	23	2·78 ± 0·42	1·39 ± 0·18
4	Gut buccal mass	24	1·37 ± 0·17	0·98 ± 0·11
5	Oesophagus	24	2·68 ± 0·25	1·63 ± 0·19
6	Mid-gut	24	2·56 ± 0·59	2·70 ± 0·40
7	Mantle	24	1·76 ± 0·29	2·31 ± 0·37
8	Kidney	27	2·24 ± 0·32	2·05 ± 0·18
9	Columella muscle	24	1·80 ± 0·34	1·17 ± 0·18
10	Female duct	24	1·03 ± 0·17	1·02 ± 0·20
11	Albuminous gland	22	1·20 ± 0·18	1·17 ± 0·18
12	Body wall	24	0·78 ± 0·08	0·94 ± 0·06
13	Dart sac	22	0·66 ± 0·07	0·59 ± 0·05
14	Foot: Fore	17	0·81 ± 0·11	0·67 ± 0·07
15	Middle	12	0·67 ± 0·08	0·70 ± 0·14
16	Rear	13	0·79 ± 0·06	0·86 ± 0·12

The results of these experiments are shown in Table 1. The rates of respiration are expressed in terms of Q_{O_2} (μl. oxygen/mg. dry weight/hr.). The tissues are arranged in order of oxygen consumption with the nervous system being the most active, then the liver, gut, mantle, kidney, followed by sundry organs with the foot having the lowest rate of respiration.

The values of Q_{O_2} showed a certain amount of variation as indicated by the size of the standard error. Much of this was due to variation between the tissues of different individuals and not due to the method of study. Thus when the liver from one animal was cut into slices and placed in eight manometers, the standard error of

the series came to 0·06, whilst the standard error on the readings on liver slices taken from twelve different animals came to 0·42.

The values we obtained for the liver ($Q_{O_2} = 2{\cdot}78 \pm 0{\cdot}42$) agree with those obtained by Baldwin ($Q_{O_2} = 2{\cdot}93$).

Table 1 shows that some of the tissues had a higher Q_{O_2} in Baldwin's solution than in Krebs's solution. The difference was most marked in the liver which was 95% higher in Baldwin's than in Krebs's, whilst the cerebral ganglia was 54% greater in Baldwin's. On the other hand, the body wall showed a Q_{O_2} that was 20% higher in Krebs's solution than in Baldwin's. This indicated that there might be a difference between the respiration of the internal and the external organs in the two solutions, the internal ones having the higher rate in Baldwin's solution and the external ones the higher rate in Krebs's. The difference between the internal and external tissues in the two solutions was found to be significant using the 't' test. The main exceptions to this generalization were the anterior part of the foot which had a higher rate in Baldwin's solution, and the mid-gut which had a higher rate in Krebs's solution.

The tissues of the intact animal are subject to varying concentrations of carbon dioxide and HCO_3^-. We studied the effect of various concentrations of CO_2 and HCO_3^- by the indirect Warburg method. We also determined the effect of adding HCO_3^- to the Ringer solution. Both methods gave essentially similar results; they led to an increase in the rate of tissue respiration. Thus use of Krebs's bicarbonate Ringer increased the Q_{O_2} from its normal value for liver of 1·39 to a value of 4·87. The gas phase was 5% CO_2, 95% O_2.

(2) *Respiration of the intact snail*

Snails were taken from the cold room and placed in the manometers. After a period of equilibration a series of readings were taken. The snails were sometimes found to respire slowly at first, but after 5 or 6 hr. the respiration increased to a 'steady' state. In fact this was not a steady level because all the snails showed considerable short-term fluctuations in their respiratory activity.

Fig. 1 shows the record of the respiration of a snail over the period of 100 min. It will be seen that the snail showed periods of high activity followed by low activity. We could not correlate these changes with either the opening and closing of the pneumostome, which was visible in many cases through the walls of the glass vessel, nor with the general muscular activity of the animal.

A series of more than one hundred readings, each of 15 min. duration, were taken over a period of 3 days, the snail remaining at 28° C. throughout this time. From these the value of the average Q_{O_2} for each snail was calculated. After the respiration had been measured, each snail was dissected and the organ systems removed, dried and weighed. A balance sheet was drawn up for each animal as shown in Table 2. The first column shows the organs in order of body weight. The body wall makes up 24% of the weight, the foot 20%, the liver 17% and the mantle collar 9%. The dry weight was approximately one-quarter of the wet weight.

Assuming that each of the tissues had been respiring at its average isolated rate

whilst it was still in the snail, the weight of each tissue was multiplied by its average Q_{O_2}. These values were then added together and equal the average oxygen consumption of all the tissues of the snail in the isolated state, i.e. the summated tissue consumption.

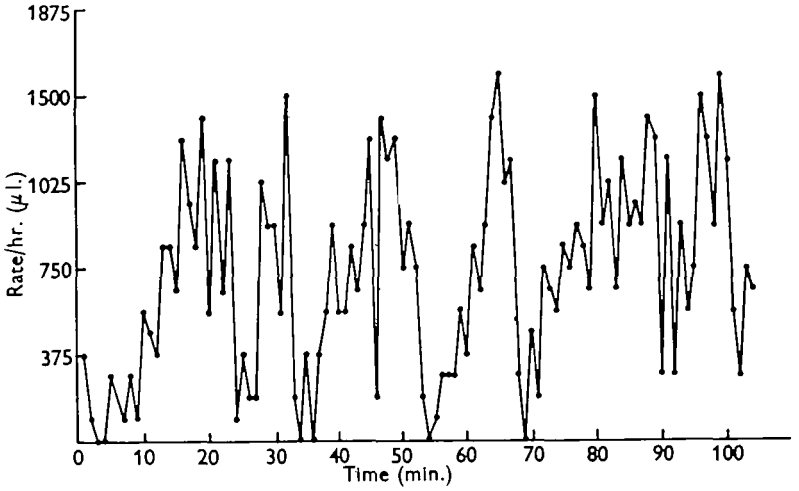

Fig. 1. The respiration of *Helix pomatia*. The rate of respiration is not constant but shows considerable fluctuations.

Table 2. *Balance sheet of tissue respiration in a single snail. For details see the text*

Tissue	Dry wt. (mg.)	Wt. × av. Q_{O_2} (Baldwin)	Wt. × av. Q_{O_2} (Krebs)
Body wall	339·0	264·0	319·0
Liver	233·0	648·0	324·0
Collar	128·6	103·0	159·0
Foot: Fore	102·1	82·2	68·1
Mid	101·0	68·7	71·4
Hind	87·6	69·5	75·4
Kidney	70·5	157·5	144·0
Female duct	68·9	71·0	70·5
Mantle	59·5	105·0	138·0
Oesophagus	39·2	101·0	64·0
Mid-gut	38·7	99·0	104·5
Buccal mass	31·8	43·8	31·2
Columella muscle	23·7	27·7	27·9
Dart sac	23·5	15·5	14·0
Albumen gland	18·5	23·9	17·0
Pedal ganglia	1·4	3·5	4·05
Cerebral ganglia	1·0	4·0	2·61
Total		1886 ± 287	1634 ± 108

The range of summated tissue consumption is indicated by the standard error for the summated average Q_{O_2} which is given by the following formula:

$$\text{S.E.} = \sqrt{\{(\text{Wt.}_{\text{liver}} \times \text{Av. rate}_{\text{liver}} \times \text{S.E.}_{\text{liver}})^2 + (\text{Wt.}_{\text{kidney}} \times \text{Av. rate}_{\text{kidney}} \times \text{S.E.}_{\text{kidney}})^2 + \ldots \text{etc.}\}}.$$

Table 3 is a summary of the balance sheet of twelve animals. The values given under the headings Q_{O_2} Krebs and Q_{O_2} Baldwin are the summated average rates of respiration of the tissues of the snails in the respective solutions.

Table 3. *Comparison between summated tissue respiration and the respiration of the intact animal. For details see the text*

Animal	Summated tissue respiration		Whole animal Q_{O_2}	$\frac{\text{Whole animal}}{\text{Summated tissue}} \times 100$	
	Q_{O_2} Krebs	Q_{O_2} Baldwin		Krebs	Baldwin
1	1698 ± 91·8	1868 ± 223	1728 (397–1950)	102	93
2	2131 ± 108	2382 ± 278	2330 (45–2770)	109	98
3	2346 ± 139	2642 ± 358	1414 (890–2080)	60	53
4	1634 ± 108	1886 ± 287	1018 (520–1520)	62	54
5	2001 ± 110	2278 ± 319	2142 (78–3070)	108	95
6	1670 ± 106	2186 ± 304	1470 (635–1770)	88	67
7	1929 ± 123	2343 ± 423	1301 (640–2240)	67	55
8	1656 ± 102	1971 ± 294	700 (390–1160)	42	35
9	1563 ± 98	1827 ± 320	925 (400–2400)	59	51
10	1388 ± 89	1687 ± 292	1090 (780–1470)	79	64
11	2127 ± 117	2527 ± 381	1062 (170–2340)	50	42
12	2091 ± 124	2395 ± 356	844 (160–2000)	41	35

The Q_{O_2} of the intact animal is the average of at least one hundred readings taken throughout 3 days. The range—given by the side of each heading—indicates the extremes at which the animal respired, but they are not the standard error of the readings nor does the average Q_{O_2} of the intact animal necessarily fall in the middle of this range. They indicate that the snail can have a very high or a very low respiratory rate. This is also demonstrated by Fig. 2 which is a histogram of the frequency at which the different rates of respiration occurred in snail 7. It will be seen that though there is quite a broad range of readings, the average is considerably below the values marked for the summated tissue respiration in either of the two solutions.

Table 3 and Fig. 2 show that the highest rate of respiration of the intact animal never significantly exceeds the rate at which the tissues could respire, and in only three out of the twelve cases cited (nos. 1, 2, and 5) is there fairly close agreement between the average rate of respiration of the intact animal and that of the isolated tissues. In the other nine cases the animals respired at 40–70% the rate of the isolated tissues. The difference is most marked in the Baldwin solution. Since the average rate of respiration of the whole animal is most frequently lower than that of the isolated tissues, and since the animal respiring at its maximum rate *can reach* the rate of respiration of its isolated tissues, there might well be some type of control of respiration in the intact animal bringing its normal range of respiration to about 60% of the possible maximum rate.

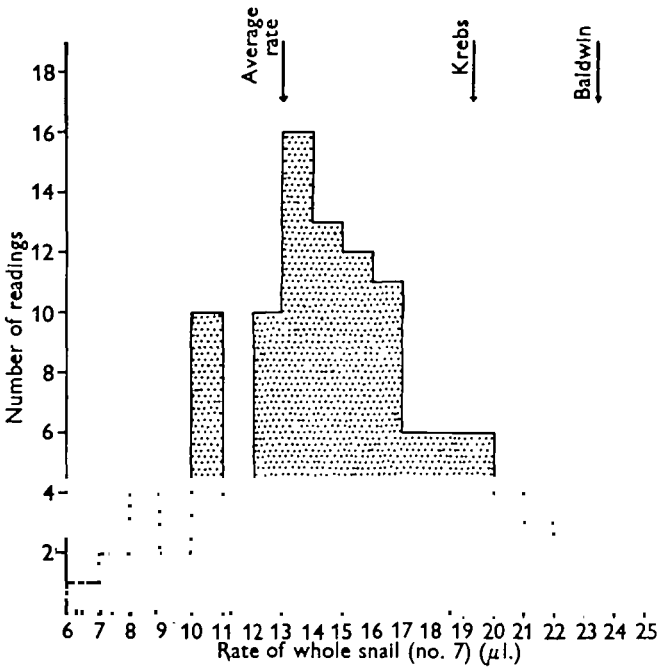

Fig. 2. Histogram of the frequency with which a snail respired at different rates. The average rate is marked for comparison with the rates at which the tissues respired in Krebs's and Baldwin's solutions.

DISCUSSION

The isolated organs of the snail each respire at a characteristic rate with the brain having the highest rate, then the liver, gut, mantle, kidney, etc. It is not unusual for the nervous system to have the highest repiratory rate in an intact animal. Thus McIlwaine (1955) pointed out that though the human brain is only 2·5% of the body weight it removes 25% of the oxygen taken in by the lungs. In most measurements on isolated mammalian tissues, however, the brain has a relatively low Q_{O_2}. Field *et al.* (1939) found the isolated organ hierarchy in the rat to be kidney, brain, liver, heart, etc. Krebs (1950), again working on the rat, found the order to be kidney, brain, liver, spleen. In both cases the brain respired at a lower rate than the kidney. McIlwaine (1955) has shown that the mammalian brain *in vitro* has a respiratory rate only 45% of the *in vivo* brain. If, however, the *in vitro* brain is stimulated electrically, the rate of respiration increases to 80% of its *in vivo* value. Such stimulation would bring the brain to a higher rate than the kidney. It is interesting to note that the isolated mammalian cortex show no electrical activity (Burns, 1951), whilst the isolated slug and snail brains show continuous electrical activity for up to 24 hr. after isolation (Hughes & Kerkut, 1956).

The relationship between tissue respiration and the basic metabolic rate has so far been analysed only for mammalian tissue. Field *et al.* (1939) carried out a respiratory balance sheet for the rat. They found that the isolated tissues respired at a lower rate than the whole animal. Thus the respiration of the whole animal was

151% of the summated tissue respiration. They suggested that if an allowance is deducted for minimal functional activity of the muscles *in vivo*, then the value for the whole animal would be some 112% the value of the isolated tissues. Martin & Furhmann (1941, 1955), working on the dog and mouse, found that the respiration of the whole animal gave a value of 112–103% of the summated tissue respiration in the dog, and 139% in the mouse.

Both these investigations differ from ours in that the isolated tissues are respiring at a slower rate than the whole animal. We, on the other hand, find that intact snails normally respire at about 60% of the tissue rate. This difference between the mammalian and molluscan situation may be explicable in terms of the blood supply to the intact tissues. In the mammals there is a well-organized blood supply carrying oxygen to within a short distance of all the cells and this is disrupted on isolation. In the snail, however, the tissues are for the most part bathed in a haemocoel and thus the isolated snail tissues are in a more normal respiratory situation than are isolated mammalian tissues.

This is supported by the fact that mammalian tissues respire better in a gas phase of pure oxygen whilst isolated snail tissues are unaffected by an increase in oxygen concentration.

Another difference between the behaviour of isolated mammalian and molluscan tissue is seen in the way in which the rate of respiration alters after the isolation of the tissue. In the mollusc the rate remains constant for the first 4 hr. after isolation. In mammals the rate falls off very markedly. Field *et al.* obtained their rates of tissue respiration at the time of isolation by extrapolation of a simple sloping curve. Lundsgaard (1950) has shown that the rate of respiration of isolated perfused mammalian tissue falls rapidly over the first $\frac{3}{4}$ hr. and then drops slowly. Field *et al.* made their first measurements about $\frac{3}{4}$ hr. after the tissue had been isolated, so in fact they extrapolated only over the gentle curve. This would then give them a low rate of tissue respiration and so explain why the respiration of the whole animals appears as 151% of the summated tissue respiration. Martin & Furhmann (1955) state: 'occasional high values of Q_{O_2} obtained for the first fifteen minutes of the experiment were not heavily weighted in plotting the results.' In one case they found that the basal metabolic rate came to 95% of the summated tissue respiration. One might expect that isolated tissue under ideal conditions should respire at a higher rate than they do in the intact animal, since this would provide a large safety factor in the working of the animal. The maximum summated tissue rate would then indicate the work potential of the animal.

In our experiments the intact snails showed a range of respiratory activity, but the maximum rate at which the animal respired never exceeded the rate at which we calculated its tissues could respire. In fact, we found that the animal was normally using only 60–70% of its maximum rate. This would indicate that the animal might have some means of controlling its tissue respiration. Barron (1943) has suggested that there may be some specific inhibitory control of intracellular reactions. He bases this view on the fact that the rate of a reaction of an isolated enzyme system is many million times faster than that of the same reaction in the

tissues and cells themselves. It is, however, difficult to tell the extent to which the rate of cellular reactions is due to inhibited, and thus controlled, systems and the extent to which the rate is due to enzyme systems diluted down with 'inert' materials.

There is some evidence for hormonal control of tissue respiration in other animals. Lardy & Maley (1954) found that thyroxin affected the rate of respiration of isolated mammalian mitochondria. In experiments where phosphorylation was controlled they found that the rate of respiration was dependent upon the thyroxin concentration, being increased by low concentrations and decreased by high concentrations.

Samuels (1956), working on the cockroach *Leucophaea*, compared the respiratory rates of isolated normal thoracic muscles and those from animals that had had their corpora allata removed 3 months previously. He found that the muscle from the allectomized animals had a 20% higher rate of respiration than had normal muscles. This would indicate that the respiration in the normal thoracic muscles is inhibited in some way by the action of the corpora allata.

On the other hand, Thomsen (1949) found that removal of the corpus allatum from *Calliphora* led to a drop in the oxygen consumption of the whole fly by 24%. Implantation of extra corpora allata led to an increase of 19% in the oxygen consumption. Samuels suggests that the difference between these results may be due to the fact that he was working on isolated tissues whilst Thomsen was working on whole animals, and that removal of the corpora allata from intact animals might lead to a lowering in the blood sugar and hence a lowering in the substrate for muscle respiration.

There is at present little or no evidence of hormone action in gastropods though there are histological studies indicating the presence of neurosecretory cells in the ganglia of *Buccinum*, *Murex*, *Nucella*, *Archidoris* and *Aplysia* (Scharrer & Scharrer, 1954; Gabe, 1953). We are carrying out a series of experiments into the action of various substances on tissue respiration in molluscs and are also determining the effects of removing various parts of the snail on the respiration of the whole animal.

SUMMARY

1. The respiratory rates of tissue slices from the various organs of *Helix pomatia* have been determined. The internal organs such as the brain and liver show a higher Q_{O_2} when immersed in Baldwin's phosphate solution. The external organs such as the mantle and collar show a higher Q_{O_2} when immersed in Krebs–Ringer solution.

2. The brain has the highest Q_{O_2}; it is followed in order by the liver, gut, mantle, kidney, columella muscle, female duct, albuminous gland, body wall, dart sac and foot.

3. The rate of respiration of a series of intact snails has been determined. The organs from each of these snails were removed, dried and weighed. From the respiration rates determined in the early part of the paper, a balance sheet was drawn up for each snail. The summated tissue respiration is compared with the respiration of the intact animal.

4. In three out of the twelve cases cited there was good agreement between the summated tissue respiration and the respiration of the whole animal. In the nine other cases the respiration of the whole animal was 40–70% below the summated tissue respiration. This would indicate the possibility that the snail has some means of controlling the rate at which the tissues respire in the intact animal.

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