THE RESPIRATORY QUOTIENT OF THE EVISCERATED SPINAL CAT.

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THE respiratory quotient of the spinal eviscerated preparation, with blood sugar maintained by infusion, has on several occasions been investigated in these laboratories. The question whether it was changed by insulin was examined by Burn and Dale [1924], at a time when it was still regarded as possible that some of the glucose disappearing under insulin might be changed directly into fat. They found that the R.Q. of the preparation was practically 1, and remained so when insulin was given. They were thus able to show that, in addition to glucose oxidized, much disappeared under insulin by change into some other substance, which could not be fat. Later Best, Dale, Hoet and Marks [1926] were able to show that the missing glucose was accounted for by deposition of glycogen in the muscles. They again, on several occasions, determined the R.Q., and found it to be 1; and calculations of the quantity of glucose oxidized, based on this figure, produced a reasonably good balance between the two sides of the account. Later again, Bodo and Marks [1928] made other determinations of the R.Q. of this preparation, prior to administration of "synthalin," and again found it to be 1.

The correctness of this determination, and of the calculations based on it has in recent years come under criticism. Kilborn [1928], working under Macleod, determined the R.Q. of spinal cats under artificial respiration, and then removed the stomach and intestines, tying off the blood supply to the liver, but keeping all other conditions constant. In particular, he maintained the same rate of pulmonary ventilation, and did not replace the supply of glucose from the liver by artificial infusion. Under such conditions the R.Q. was again determined and was found usually to be in the neighbourhood of 1, though individual samples gave values as much in excess of this as 1·8. Since the respiratory rate and volume were unaltered, though the rate of absorption of oxygen fell

to as little as one-quarter of that observed before the evisceration, Kilborn concluded that the R.Q.'s which he thus obtained were artificial; and we find no reason for questioning this conclusion as regards the results in general. It is difficult, however, to reconcile all his data with the view that the immediate and principal cause of the rise of the R.Q. with evisceration was simply the blowing off of CO_2 in excess of its metabolic production. It would be expected, under such conditions, that the error would chiefly affect the period immediately following evisceration; whereas in most of Kilborn's experiments the rise of the respiratory quotient above 1 takes place towards the end of the experiment, where the metabolic rate has become very low, and it may be suspected, in the absence of records of the blood-pressure, that the circulation was becoming seriously deficient. However that may be, we agree with Kilborn that no satisfactory data as to the real R.Q. of the eviscerated preparation can be obtained by such a method. It is not so easy to follow the assumption that the quotient determined before evisceration may be taken as the real one after that operation, and that the extra volume of CO_2 calculated to produce the apparent quotient, on that assumption, may be accepted as that of the CO_2 blown off in excess of metabolic production. This calculation is used by Kilborn, and later by Ferguson, Irving and Plewes [1929], who endeavour, admittedly without complete success, to discover the source in the body of the large amount of CO_2 needed to justify this hypothesis.

These writers appear, further, to assume that the quotients used as a basis of calculation in this laboratory were obtained by a method subject to the error which they detected in their own. This is not strictly justified; though we realize that the details hitherto published have not been sufficient to exclude it as a possibility. None of the eviscerated preparations used here for quantitative investigations has, in fact, been subjected to artificial respiration at the same rate as that applied before evisceration. As a matter of practice, after destruction of the brain under anæsthesia, the other operative procedures, up to the completion of the evisceration, have been carried out under artificial respiration by simple inflation with a pump, the chest expelling the air by natural collapse, through a side-opening on the tracheal cannula, between the positive strokes. Immediately after completion of the evisceration, however, the preparation has been transferred to the Schuster double respirometer; the tracheal cannula has been replaced by a Y-shaped tube, through one limb of which the chest is inflated from one chamber of the double pump, the same volume being withdrawn from it by suction

on the return stroke into the other chamber. Such a method enables efficient ventilation to be carried out with a relatively small movement of the chest, and is, in our experience, much. more favourable to the maintenance of good circulation in this preparation than the use of positive ventilation only. The necessary stroke volume was further reduced by enriching the air of the closed respirometer system with oxygen.

Another difference between our procedure and that used by Kilborn and the other Toronto workers is in the supply of carbohydrate. We have always removed the kidneys and kept up the blood sugar by continuous infusion of glucose. They apparently allowed the blood sugar to fall naturally after removal of the liver from circulation, keeping the kidneys intact and presumably functional, and not making good from outside the glucose used or excreted. Bornstein [1929], using dogs under chloralose anæsthesia, so that no question of excessive artificial ventilation arose, found that, after evisceration, the R.Q. rose to and remained at 1 if the blood sugar was kept up by infusion, but fell to a lower value if the supply was allowed to be naturally exhausted.

For these various reasons it did not appear to us that the Toronto investigations justified the conclusion that the R.Q. in experiments on the eviscerated preparation, carried out under our conditions, was significantly less than 1; still less that it could be assigned the value observed before evisceration. The point was one of importance, however, not only for our past conclusions, which have been called into question by Macleod [1929] on the basis of Kilborn's work, but also for further experiments which we had in hand. We decided, accordingly, that it ought to be investigated more critically than before, and with a special view to the possibilities suggested by Kilborn and others. They do not appear to have attempted to determine experimentally the true R.Q. of the spinal eviscerated preparation, without excessive ventilation. We have made further experiments with this special object in view.

METHODS.

Our former determinations of the R.Q. on this preparation were made by use of the complete Schuster respirometer, which automatically registers the rate of oxygen consumption by volume, the carbon dioxide output being determined gravimetrically by absorption in soda-lime tubes during sample periods, and translated into volume by calculation. We have recently used this method again, with similar results to those

earlier obtained. For experiments made with the primary object of investigating the R.Q., however, it seemed more convenient to collect the expired air for analysis in Douglas bags. This had the additional advantage of conformity with Kilborn's method. We adhered, however, to the use of the two-chambered pump of the Schuster apparatus, for reasons already given. The first chamber drew air from the room, warmed by passing through a heated cylinder, and pumped it into the lungs, from which at the return stroke the same volume was drawn into the second chamber, and passed from this at the next stroke into the collecting bag. In the first experiment of the series, the results of which are given below in Table I, it will be seen that the R.Q. was first determined during two periods by the use of the respirometer and absorption tubes, and then during a third period, without other change, by the Douglas bag and gas analysis, without significant difference in the results.

Throughout the series of experiments here recorded the pump was run at a uniform rate of about 20 strokes per minute. The total ventilation rate was accordingly determined by the stroke volume, which was kept constant in each experiment, but differently adjusted for each. The adjustment of the ventilation rate to that of the metabolism obviously needed attention for our purpose. It was not easy, however, to find any quantitative indication that it was adequate and not excessive. Preliminary trials showed us that it could not safely be reduced to as little as 100 c.c. per kg. per minute; under such conditions the oxygenation of the arterial blood became obviously defective and the arterial pressure began to fall rapidly. After a number of trials we found no better method than to adjust the stroke to such a volume that the circulation was well maintained. This resulted in one rate as low as 121 c.c., and one as high as 231 c.c. per kg. per minute; the majority, however, ranged from about 140 to 170 c.c.

When the operation had been completed, the preparation attached to the double pump and the requisite adjustments made, a cannula was tied into the jugular vein and glucose infused at a slow constant rate as in all the earlier experiments. The preparation was then left to itself for a period of an hour or more, to eliminate all possible irregularities due to the change of ventilation rate after operation, before the collection of expired air was begun. The contents of each bag were carefully mixed before drawing the sample for analysis by the large Haldane apparatus. Samples of the "inspired" air, as it entered the pump chamber, were also taken and analysed. The table given by Cathcart [1918] was used

TABLE I.

No.	Date	Cat wt. (kg.)	Ventilation (min. vol. c.c.)	Glucose infusion (g. per hr.)	Metabolism periods	CO₂ c.c.	O₂ c.c.	R.Q.	Blood samples time	Plasma CO₂ capacity	Blood sugar
1	29. i. 30	4·0	484 = 121 per kg.	0·45	1.35–2.5 (Tubes)	370	336	1·1	1.35	24	0·390
				0·6	2.15 (Insulin 20 units)	—	—	—	2.10	30	0·248
					2.50–3.50 (Tubes)	611	501	1·2	3.0	24	0·235
					4.0 (Douglas bag)	—	—	1·1	4.0	26	0·240
					Douglas bag experiments in which outside air was analysed. (CO₂, O₂ in p.c.)						
2	13. ii. 30	3·1	530 = 171 per kg.	0·45	1.37–2.37	—	—	1·4	1.27	24	0·464
					4.2–4.32	1·33	1·29	1·03	2.42	24	0·464
									4.44	20	0·384
3	14. ii. 30	3·6	520 = 144 per kg.	0·45	1.37–2.37	1·30	1·33	0·97	1.22	21	0·464
					3.50–4.43	1·08	1·09	0·99	2.40	19	0·464
									3.53	21	0·478
									4.46	20	0·460
4	18. ii. 30	2·9	405 = 140 per kg.	0·3	1.26–2.26	2·24	2·23	1·0	1.13	—	0·310
					3.0–4.30	2·00	2·14	0·92	2.26	17	0·248
									4.30	17	—
5	19. ii. 30	2·6	405 = 155 per kg.	0·3	1.30–2.55	1·90	1·92	0·99	1.10	20	0·212
									2.50	23	0·212
6	25. ii. 30	3·5	520 = 149 per kg.	0·45	12.45–1.45	2·18	2·19	0·99	12.40	42	0·354
				0·60	2.4 (Insulin 20 units)	—	—	—	2.0	32	0·354
					2.37–3.37	2·00	2·14	0·92	3.40	—	0·300
7	27. ii. 30	3·0	520? = 173 per kg.	0·42	1.30–2.35	1·77	1·89	0·92	1.27	—	0·398
				0·48	2.45 (Insulin 20 units)	—	—	—	2.36	24	0·444
					3.18–4.18	1·66	1·78	0·91	3.20	26	0·420
									4.23	—	0·251
8	11. iii. 30	2·7	624 = 231 per kg.	0·36	1.18–2.18	1·45	1·66	0·84	1.15	—	0·444
				0·54	2.22 (Insulin 20 units)	—	—	—	2.10	24	0·528
					2.52–3.52	1·20	1·31	0·90	2.50	16	0·312
									3.55	—	—
9	14. iii. 30	3·95	600 = 152 per kg.	0·51	12.50–1.45	2·01	2·25	0·87	12.47	36	0·212
					1.55 (Adrenaline infused)	—	—	—	1.53	36	0·220
					2.30–3.25	2·80	2·94	0·95	2.25	32	0·220
									3.34	38	0·212

in correcting "the apparent R.Q." for the altered nitrogen content of the expired air.

RESULTS.

The results are given in Table I. An inspection of the figures in detail will show that there is no general indication of a R.Q., so high as to be obviously fallacious, being produced by excessive ventilation. In the first period of Exp. 2 there is, indeed, a quotient of 1·4 recorded. The figure is unique in the whole series, and it is unfortunate that, though the experimental readings, from which the percentages of oxygen and carbon dioxide and the R.Q.'s were calculated, were preserved and checked in all subsequent experiments, they were mislaid in this instance. We suspect an arithmetical error; but, being unable to prove it, we produce the figure as it stands in our notes. The later sample, taken after an interval of 1½ hours from the same experiment, gives a R.Q. of practically 1. It should further be noted that, neither in this nor in any other case, is there any evidence of a serious depletion of the alkali reserve of the plasma during the course of the experiment.

It will be seen that the percentages of CO_2 in the expired air vary in the different experiments. There is, however, no correspondence between a low proportion of CO_2 and a high ventilation rate. The lowest, in Exp. 3, is obtained with a minute ventilation of only 140 c.c. per kg. It is, indeed, impossible in any one experiment to predict from the weight of the cat what the rate of metabolism will be. Ideally, no doubt, the rate of artificial ventilation should be adjusted by trial to produce a nearly constant percentage of CO_2 in the "expired" air. Our object, however, was not merely to obtain the value of the quotient under ideal conditions, but to test more fully the effect of the conditions under which the earlier R.Q.'s were obtained, which have been criticized as due to over-ventilation. Our figures show that in none of our experiments did the percentages of CO_2 expired, or of oxygen absorbed, fall to the levels at which the high quotients were obtained by Kilborn in the later stages of some of his experiments, shown in his Table II. According to that table, the periods in which Kilborn observed these high quotients were characterized by very low values for percentages of oxygen absorbed, as compared with the earlier periods of his own experiments, as well as with any of ours—0·59 p.c. with R.Q. = 1·45, 0·82 p.c. with R.Q. = 1·34, 0·68 p.c. with R.Q. = 1·23.

These low metabolic rates in the later stages of his experiments strongly suggest that the circulation was becoming ineffective. In our

own experiments there has generally been some decline of metabolic rate as the arterial pressure gradually fell; but we have never encountered such a sudden and drastic reduction as Kilborn's records suggest. If we had done so, we should not have measured the quotient during such a terminal period.

Turning to the quotients, the determination of which was the object of our experiments, it will be seen that in six out of the nine experiments the figure obtained at some stage of the experiment was 1, or so nearly 1 that the difference cannot be regarded as significant. In two of these experiments, Nos. 4 and 6, it subsequently fell to a corrected value of about 0·92, in the latter case after a large dose of insulin; and in another experiment, No. 7, it had about this value, both before and after insulin. In one of the earlier determinations, using the circulating respirometer (Exp. 11 of Burn and Dale), a value as low as this was obtained after insulin, in a preparation which showed R.Q. = 1·02 at an earlier stage; but it was the only quotient in that series seriously different from 1. In the last two experiments, Nos. 8 and 9, we recorded quotients substantially lower than in any of the others. In the former it rose after insulin, in the latter still more after adrenaline. In view of the nature of the effect of adrenaline on muscle glycogen, with which two of us are dealing in a separate paper, this latter rise was to be expected, owing to the production of lactic acid from muscle glycogen. At the same time, it will be seen that there was no fall in the alkali reserve of the blood plasma, such as might have been expected to accompany such an effect.

One further point, of special importance for our purpose, should be noted. Exp. 8 is not only the one showing the lowest R.Q., but is also the one experiment of the whole series in which the rate of ventilation may be suspected of exceeding the metabolic requirement to the extent of those used by Kilborn. This exceptionally low quotient being associated with an exceptionally high rate of ventilation, it is difficult to attribute the more frequent quotients in the neighbourhood of 1 to excessive removal of CO_2 by the milder ventilation used in them. Nor is any support for such a supposition to be obtained from the figures for percentage of CO_2 in the expired air. This falls in Exp. 8, with the low quotient, to a lower level than in any save one (No. 3) of the other experiments.

DISCUSSION.

These results contain irregularities which we did not expect, and which we cannot wholly explain. We have considered the possibility that the later experiments, in particular, may have been vitiated by a loss of CO_2, without finding any reason for supposing that it occurred. An inspection of the figures will make it obvious that the method is one which allows only a very narrow margin of error; but we have no reason, apart from their discordance with the majority of the results obtained in this series, and with the earlier ones obtained by another method, to regard such error as more likely to have occurred in the experiments giving the low R.Q.'s. On the other hand, we have failed to find any reason for assuming error, from excessive ventilation or any other cause, in the experiments showing quotients in the neighbourhood of unity.

In such circumstances we have to admit two possibilities of interpretation. The first is that the spinal eviscerated preparation, with maintained blood sugar and treated in other respects according to our routine, has commonly a true respiratory quotient of 1, but that instances occur, either during part or the whole of an experiment, in which it may be about 0·92, or in rarer cases as low as about 0·85. In that case, with no indication of conditions previous to or during the experiment which determine this variation, it is impossible to predict when it will occur. We should accordingly have to conclude that calculations such as those made by Best, Dale, Hoet and Marks [1926] would be liable to an additional factor of uncertainty. Their assumption of a quotient of 1 would remain the most probable, and its incorrectness in every case would be very unlikely; but the possibility of its not being accurate in every case would have to be admitted.

This is, of course, a very different proposition from that advanced by Kilborn, accepted by others, and used in criticism of the aforesaid calculation. According to their view, the true R.Q. in all our experiments would be similar to that of the preparation before evisceration, viz. 0·71 to 0·8 in Kilborn's own series; and it would have to be assumed that the predominance, in these experiments, of quotients so near to unity, is due to our habitual success in providing just the appropriate degree of excessive ventilation. Neither in our own results nor in the details of Kilborn's own records can we find any justification for such an assumption.

The second possibility is, with frank recognition that the determination is not an easy one to make with consistent accuracy, to suppose that

some unrecognized source of error has entered into either the majority or the minority of our experimental determinations, and that either the more frequent value 1, or the exceptional value of about 0·85, represents the true quotient in all. We should then have to appeal to collateral evidence for an indication as to which was the more probable. Best, Dale, Hoet and Marks [1926] published one attempt to calculate the relation between glucose disappearing, from all sources, and oxygen consumed in the spinal eviscerated preparation without insulin. They found a remarkably close agreement between the two values. In a separate publication by two of us (C. and M.) [1930] three more such experiments without insulin are published. The calculation is a relatively simple one; no serious change of muscle glycogen being in question, the only important items on the one side of the account are glucose infused and glucose leaking from the *cul-de-sac* liver, and on the other side oxygen consumed. Now if the assumption is made that the quotient is 1, so that the oxygen is used in burning carbohydrate only, the two sides of the account show a balance which is sometimes almost too exact, and in no case differ by more than 10 p.c. of the total; and such differences are always in the direction of showing a little more glucose lost than the oxygen will account for. If the R.Q. were taken as 0·85, we should have to assume that, in these experiments without insulin or adrenaline, at least one-half of the glucose lost had not been oxidized, but had vanished beyond recognition. In other words, the assumption of a real quotient even as low as 0·85 involves us in a further assumption of unknown possibilities, having no relation to the experimental facts. We have already mentioned Bornstein's experiments on anæsthetized eviscerated dogs; many years earlier Porges and Salomon [1910] had shown that, in dogs rendered diabetic by a preliminary pancreatectomy, ligaturing off the rest of the abdominal organs under urethane caused the R.Q. to rise to a value of approximately 1. Boothby, Mann and Magath [1928], in hepatectomized dogs breathing normally, found a R.Q. of 0·85 to 0·95, rising to a higher level, and even to 1, when an adequate amount of glucose was given. When it is further remembered, that feeding with a diet rich in carbohydrate may produce, in a completely normal animal, with all its organs intact, a quotient approximating to and even reaching unity (cf. Chaikoff and Macleod [1927]), there seems to be such a weight of probability in favour of this value for the quotient of our preparation, consisting essentially of muscles supplied with abundant glucose, that overwhelming direct evidence would be required to establish a lower value as the true one.

For these various reasons, and in spite of the relatively few exceptions in our own direct measurements, we are still of opinion that, when a quotient has to be assumed, in calculation from respiratory data obtained with the eviscerated preparation supplied with glucose, the only reasonable and safe assumption is to take it as unity.

We may add that this is a purely practical conclusion in relation to special conditions, and has no bearing on the more fundamental question as to whether muscle always supplies its energy requirement by oxidation of carbohydrate alone. Burn and Dale [1924] incidentally mentioned that their data were compatible with such a view, which was being advocated on other grounds at the time of their publication. They did not suggest, and we do not, that results obtained under such limited conditions contribute positively to the settlement of a much wider question.

SUMMARY.

The R.Q. of the eviscerated spinal preparation, with artificially maintained blood sugar, has been determined under conditions excluding excessive ventilation, and found usually to be 1. In certain cases somewhat lower quotients were obtained, but the view that the usual quotient of 1 is due to excessive ventilation is shown to be untenable.

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