

(From the Laboratory of Zoophysiology. University of Copenhagen.)

## On the Relation between the Temperature and the Respiratory Exchange in Fishes.

By

**Richard Ege** and **August Krogh** (Copenhagen).

Mit 3 Figuren im Text.

In a series of papers one of us<sup>1)</sup> has published the results of researches concerning the influence of temperature upon the rate of development of embryos of animals belonging to widely different groups and of chrysalides of insects. In all these cases it was found that the processes examined could not be expressed even approximately by the well known formula of van't Hoff, which will express approximately the relation between the temperature and most chemical reactions and which has been generally supposed to be applicable also to biological phenomena of the most divers kind<sup>2)</sup>.

According to van't Hoff's formula the velocity of a process increases in geometric progression when the temperature is increased in algebraic progression and we have  $V_{t+10} = V_t \cdot Q_{10}$  or as the formula is generally used  $V_{t+100} = V_t \cdot Q_{10}^{10} = V_t \cdot Q_{10}^{10}$  where  $Q_{10}$  or  $Q_{100}$  is a constant.

---

<sup>1)</sup> A. C. Johansen and A. Krogh, The Influence of Temperature and certain other Factors upon the Rate of Development of the Eggs of Fishes. Conseil international pour l'explor. de la mer. Publications de circonstance, No. 68, 1914. Krogh, On the influence of the temperature on the rate of embryonic development. Zeitschr. f. allg. Physiologie, 1913, 16, 163. Krogh, On the rate of development and CO<sub>2</sub> production of chrysalides of *Tenebrio molitor* at different temperatures. Ibid., p. 178.

<sup>2)</sup> Kanitz, Zeitschr. f. Elektrochemie 1907, 13, S. 707.

Snyder, Amer. Journ. of Physiol. 1908, 22, p. 309.

The development velocities examined were found however to conform to a much simpler formula  $V_{t^0+1^0} = V_{t^0} + k$  in which  $k$  is a constant over a considerable range of temperatures.

When expressed in curves the second formula gives temperature — velocity curves which are straight lines, while a curve corresponding to van't Hoff's formula is at low temperatures nearly parallel to the axis of abscissa, turns upwards slowly at first and then more rapidly and ends by being nearly vertical.

The results of the development experiments made it desirable to see whether other biological processes did really conform to van't Hoff's formula, as alleged, or if some other mathematical expression would

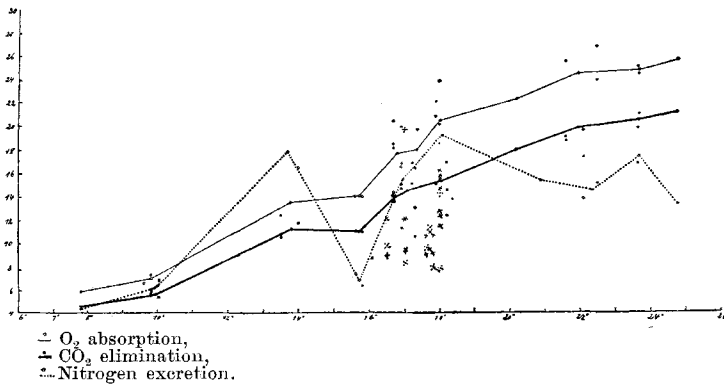


Fig. 1.

suit the facts better. As the material which could be found in the literature was not for the most part accurate enough to warrant the construction of definite smooth curves, renewed experimentation became necessary, and this was of course directed against such processes with regard to which the available material pointed towards simpler temperature — velocity relations. Among these was the respiratory exchange of fishes.

The influence of the temperature upon the respiratory exchange of fishes has been examined in Zuntz' laboratory by Knauthe and the results have been summarized in the curves Fig. 1<sup>1)</sup>. The curves representing the relations between oxygen absorption and

<sup>1)</sup> Cronheim, Zeitschr. f. Fischerei 1911, 15, S. 336.

temperature or  $\text{CO}_2$  elimination and temperature are certainly not smooth nor very definite, but it is obvious that if a smooth curve should be drawn to represent the experimental results as nearly as possible that must be a straight line or very nearly so.

We have made a series of experiments on a goldfish weighing 9,3 g which was during all the experiments in a state of complete inanition. In almost all the experiments the fish was brought to the desired temperature  $\frac{1}{2}$  to 1 hour before the determinations began. These were made by enclosing the fish in a stoppered bottle of 2700 cc capacity, filled completely with water, which had been shaken with air at a slightly higher temperature than that at which the experiment should be made<sup>1)</sup>. The oxygen content of the water was carefully determined by Winklers titration. The bottle was then left in a constant temperature waterbath for a suitable time varying from 157 minutes at the highest temperature ( $25,7^\circ$ ) to 4229 m in one experiment at  $0^\circ$ . At the end of the experiment 3—4 samples of the water were taken and analysed.

We have taken care not to let the oxygen percentage of the water sink so far that there could be any danger of oxygen want. The  $\text{O}_2$  tension of the water has in no case been lower than 11% or 80 mm. Winterstein has found 16 mm tension as the limit above which want of oxygen does not occur<sup>2)</sup>.

We have avoided the influence of bacterial growth by cleaning the bottle carefully and by using water which if not sterile contains only very few bacteria. We have not been able to detect any influence of the length of the experimental period upon the result<sup>3)</sup>, no doubt because our fish was always extremely quiet.

We have found finally that in the 22 days, during which the experiments were made, the gas exchange of the fish at a constant temperature remained practically unaltered (Table 1, exp. 2 and 13, 3 and 12). The results of the single experiments are therefore directly comparable and require no reduction whatever.

The results of this series of experiments are given in table 1 and plotted graphically in fig. 3 p. 54.

---

<sup>1)</sup> We used a slightly higher temp. to be sure to avoid the formation of air bubbles in the water during the experiment.

<sup>2)</sup> Pfl. Arch. 125, p. 73.

<sup>3)</sup> Lipschütz, Zeitschr. f. allg. Physiologie 1911, 12, p. 85 and 117.

Table 1.

No.	Date	Duration minutes	Temp	O <sub>2</sub> per l initial samples cc	O <sub>2</sub> per l final samples cc	O <sub>2</sub> absorbed per l water cc	O <sub>2</sub> absorbed per kg and minute
1.	7. II.	450	14,4	7,24 7,43	5,69 5,68 5,59	1,69	1,09
2.	8. II.	300	20,1	6,19 6,08	4,29 4,20 4,31 4,26	1,87	1,81
3.	11. II.	252	25,7	6,04 6,03	3,51 3,46	2,55	2,94
4.	12. II.	185	25,5	5,54 5,54	3,65 3,64 3,69	1,88	2,95
5.	13. II.	157	25,7	5,68 5,62 5,67	3,95 3,97 4,02	1,68	3,11
6.	15. II.	392	14,9	6,81 6,87	5,32 5,35 5,29	1,49	1,10
7.	18. II.	296	15,0	7,16 7,28	6,03 5,86 6,08	1,23	1,21
8.	19. II.	592	7,9	8,32 8,35 8,35 8,48	7,46 7,48 7,31	0,93	0,471
9.	19. II.	777	7,9	7,83 7,85	6,26 6,22 6,26	1,59	0,593
10.	20. II.	2479	0,0	8,00 7,95	7,08 6,99 6,99 7,00	0,96	0,112
11.	23. II.	4229	0,0	8,29 8,34	6,75 6,77 6,74	1,53	0,108
12.	27. II.	192	24,8	5,68 5,72	3,73 3,75 3,81	1,94	2,93
13.	28. II.	200	20,1	6,05 6,07	4,78 4,74 4,81	1,28	1,86

Though the fish was extremely quiet in all the experiments we thought that the results might be influenced to a certain extent by

the muscular movements which would tend to increase the metabolism and probably comparatively more by higher temperatures than by lower. It is obvious that if the temperature influence is to be studied quantitatively the disturbing factors must be eliminated as far as possible, and in most animals the „voluntary“ muscular movements constitute certainly the most important disturbing factor and that which is the principal cause of fallacy.

We performed therefore a second series of experiments in 0,48% solution of urethane<sup>1)</sup> in which the fish is narcotized practically completely, the only visible movements being respirations made at long intervals. As these were not deemed sufficient to ensure the

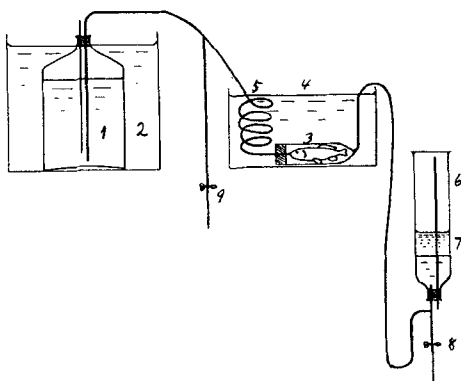


Fig. 2.

necessary supply of oxygen for the gills we had to have recourse to an arrangement for artificial respiration. This was provided in the manner shown in Fig. 2.

A large bottle (1) was filled with 0,48% urethane which was carefully saturated with air at the temperature of the bath (2), in which it was kept during the experiments. The fish was enclosed in a small vessel (3) and a current of the urethane solution was led into the mouth through a cannula. The solution was first brought to the temperature of the bath (4) by passing through a long lead tube (5), immersed in the water. From the fish the solution passed on to an inverted measuring flask (6), in which it was collected

---

<sup>1)</sup> We convinced ourselves by special experiments that the presence of urethane did not in the least interfere with the oxygen titrations.

below a thick layer of paraffin oil (7). The flask was lowered at frequent intervals during each experiment in such a way that the surface of the oil was kept at a practically constant level, and a uniform current of water through the mouth and gill cavity of the fish was thereby insured. The time corresponding to a flow of 300 cc was carefully measured, and when a little over 300 cc had passed into the flask the water was allowed to run out nearly completely through the tube (8), the flask being raised meanwhile so that the absolute level of the oil remained unaltered. Of the water running out about 200 cc were collected as a sample and titrated for oxygen. The water from the bottle (1) was sampled several times through the tube (9) and likewise analysed.

The temperature of the bath (4) and the fish was changed from time to time and the determinations were begun 20–60 m after each change. A uniform current of water was of course maintained also before the determinations, and at each temperature the flow was regulated in such a way that approximately  $\frac{1}{5}$  of the oxygen originally present in the water should be used up by the fish.

The results are given in the following table and plotted in Fig. 3.

Table 2.

No.	Rate of flow 300 cc in m	Temp.	O <sub>2</sub> per l initial samples cc	O <sub>2</sub> per l final samples cc	O <sub>2</sub> absorbed per l water cc	O <sub>2</sub> absorbed per kg and minute cc
1.	23,5	18,0	6,24 <sup>1)</sup>	4,80	1,43	1,69
2.	22,5	18,1		4,79	1,44	1,73
3.	30,0	14,4		4,90	1,34	1,21
4.	34,0	10,3		5,15	0,993	0,789
5.	31,05	10,5		5,33	0,904	0,789
6.	20,37	19,1		5,08	1,15	1,53
7.	20,53	19,2		5,06	1,18	1,56
8.	14,73	23,1	5,40 <sup>2)</sup>	4,11	1,29	2,36
9.	14,93	23,2		4,06	1,34	2,42
10.	9,93	28,0		4,16	1,24	3,43
11.	9,73	28,1		4,13	1,27	3,53

It is at once apparent that the results obtained on the narcotized fish conform pretty closely to the curve which represents the in-

<sup>1)</sup> Average of three determinations taken at intervals: 6,24, 6,22, 6,25.

<sup>2)</sup> A fresh bottle with urethane solution. The O<sub>2</sub> determinations gave 5,43, 5,37.

fluence of temperature upon the oxygen absorption of the normal fish. The slight muscular movements made by the latter have not therefore had any measurable influence upon the respiratory exchange.

The curve obtained is not a straight line. On the other hand it cannot be expressed satisfactorily by the rule of van't Hoff since

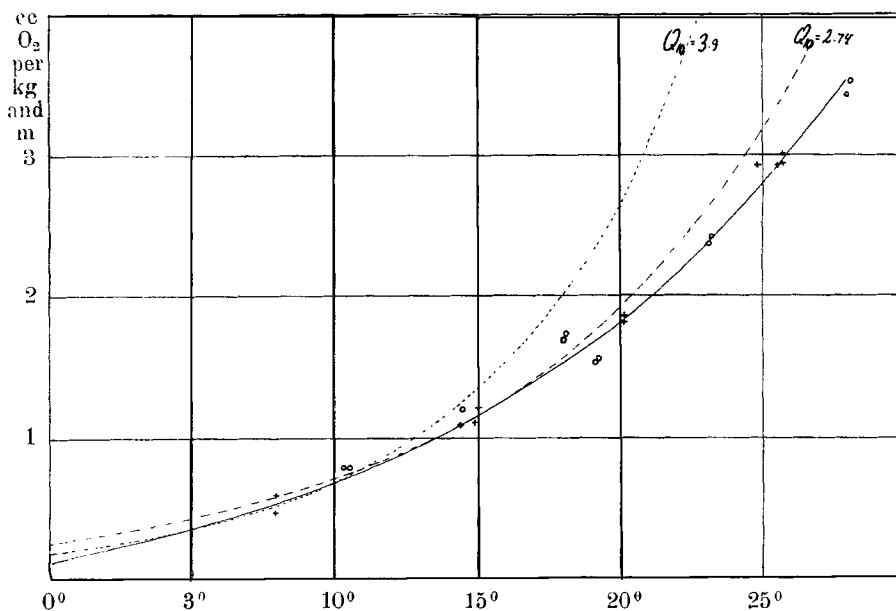


Fig. 3. + Normal fish. o Narcotized fish.

$Q_{10}$  shows a steady and very considerable decrease from  $0^\circ$  to  $28^\circ$ .

We find

$0^\circ - 5^\circ$	$Q_{10} = 9,8$
$5 - 10$	3,8
$10 - 15$	2,9
$15 - 20$	2,45
$20 - 25$	2,35
$23 - 28$	2,2

The "average  $Q_{10}$ " as it is very often calculated from a similar series of figures works out as 3,9 and if we construct the curve corresponding to this and touching the real temperature — resp. exchange curve at  $10^\circ$  we obtain the one named  $Q_{10} = 3,9$  in Fig. 3. If however the value 9,8 is excluded as being "obviously erroneous"

the average of the rest becomes 2,74, and when the corresponding curve is constructed to touch the real one at 15° the result is the curve  $Q_{10} = 2,74$ . Neither the one nor the other can express the experimental results at all satisfactorily.

Discrepancies of the same order as those met with in the present case between the observations and the van't Hoff curves, which are supposed to represent them, are the rule and not the exception with regard to biological processes. It has been stated in fact by one of the champions of van't Hoff's rule as applied to biology that at lower temperatures a higher  $Q_{10}$  is invariably found<sup>1</sup>). This is however explained or explained away in various ways, and the obvious conclusion that the processes in question do not follow the rule is disregarded on almost all sides<sup>2</sup>), presumably because the conception of the biological processes as being of a purely chemical nature is too tempting. It is of little use however that a conception is beautiful, when it cannot and will not agree with the facts, and it is certainly not too much to say that the better the facts are observed the less agreeable will they be found to be.

It will be shown in a subsequent paper by one of us that the relations between temperature and respiratory exchange, which we have established for a fish, are identical with those obtaining for frogs, when the influence of muscular activity, which is much more considerable in frogs than in goldfishes, has been eliminated.

Similar experiments are being extended to other groups of animals and a comprehensive theoretical treatment will not be attempted before the whole material is available.

---

<sup>1</sup>) Snyder.

<sup>2</sup>) There are some exceptions however. Knowlton and Starling (Journ. of Physiol. 1912, 44, p. 217) point out with regard to experiments on the heart rate that  $Q_{10}$  will vary continuously with the temperature. "It seems therefore misleading to speak of a temperature coefficient for the heart".