OXYGEN CONSUMPTION OF ANIMALS AND TISSUES AS A FUNCTION OF TEMPERATURE*

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ABSTRACT

The generally accepted view that rates of oxygen consumption of tissues and poikilotherms increase regularly with rising temperature was subjected to careful examination using brain slices and skin of rats and nine different species of aquatic and terrestrial animals. It was found that, although there are statements in the literature to the contrary, the influence of temperature is a regular one and respiration increases with rising temperature so that when rates of oxygen consumption are plotted against temperature the resulting curve is regular without dips or peaks except the maximum expected at the optimum temperature.

In 1916 Krogh summarized his own and others' work on the effect of temperature on metabolism: ". . . the velocity of the catabolic reactions increases in all animals with rising temperature up to a maximum at and above which the temperature has a deleterious effect on the organism. The maximum temperature probably differs considerably for different animals . . . and the more rigorously standard conditions are maintained the more regular is the influence of temperature observed." All students of temperature effects except a very few (O'Connor, 1939, 1942 *a*, 1942 *b*, 1947, 1950, 1953, 1955, 1957; O'Connor and McKeever, 1950; O'Connor and O'Donovan, 1942) still agree that metabolism, when not modified by central regulation, increases with rising temperature and that, although it increases to a different degree in different animals and tissues, the rise is a progressive one, so that when rates of oxygen consumption are plotted against temperature the resulting curve is regular without dips or peaks. O'Connor (1955), however, states: "The traditional conception of the influence of rising temperature on oxygen consumption is in error. The increase is not continuous but is interrupted by several pronounced falls . . . if these identical observations had been collected without a systematic sequence of temperatures the peaks would not be obvious. If their existence were suspected and an attempt made to estab-

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lish them by accumulating more observations, even on the same animal, the shifts in the peaks would so obscure them that no relationships between oxygen consumption and temperature could be recognized except a positive correlation with a large and inexplicable variance . . . this is the result obtained by Field, Fuhrman, and Martin (1944) on rat brain slices."

We have therefore re-examined this problem, using very small temperature changes over a wide range and using single animal or tissue samples each time a range was covered.

Methods

Animals.—Oxygen consumption rates were measured in respirometer vessels fitted to Warburg manometers and using the carboysyringe device described by Burk, Hobby, and Hunter (1957). The vessels were of several sizes and shapes with volumes ranging from 4 to 20 cc. so that each animal used occupied a large part of the total volume of the vessel. The gas phase was 100 per cent oxygen and carbon dioxide was absorbed with 5 N KOH. Marine animals (collected near Hopkins Marine Station) were rinsed and placed in an appropriate quantity of filtered sea water and terrestrial animals were placed on moist filter paper. Rate of oxygen consumption (QO_2) is expressed as microliters of gas consumed per milligram wet weight per hour. The temperature of the surrounding water bath could be very rapidly changed by adding about one liter of boiling water and resetting the temperature with a Thermonitor (E. A. Sargent and Co., Chicago). Regulation was within ± 0.01°C. A series of determinations was made on single animals at increasing temperatures from several degrees below that of its natural habitat to several degrees above. The temperature intervals used were from ½ to 2°C. and all data were recorded on single animals so that any "peaks" in metabolic rate would not be obscured by averaging data. By using many animals in this way, each over a somewhat different and overlapping range of temperatures, the entire relationship becomes apparent. Since it was possible that the metabolic rate of an animal could decrease with time, we always increased rather than decreased the environmental temperature. If one worked with decreasing temperatures, a decreasing QO_2 would not necessarily be a temperature effect. Each temperature at which a QO_2 was determined was maintained constant until the QO_2 was constant, usually 20 to 40 minutes, and then the bath temperature was raised slightly and another determination of QO_2 made. The internal temperature of the poikilotherm cannot be considered identical with that of the environment unless great patience is exercised to allow sufficient time for this to occur, even when the change in surrounding temperature is only about 1°. The animal was in no sense acclimated to the new temperature, since this process requires much longer. Although the vessel size was carefully selected for each animal, it was possible for it to move within the vessel. If the animal became active (this could be observed through the glass vessel) readings were not taken until it was quiet again. Thus, a standard, resting metabolic rate was obtained for each temperature. Replicate determinations at any one temperature agreed well.

Tissues.—Tissues were from male rats of the Wistar strain. Foot skin was prepared as previously described (Fuhrman and Fuhrman, 1957) and slices of cerebral cortex were obtained with a template. Suspension medium was Krebs-Ringer-phos-

phate (Cohen, 1957) and the gas phase 100 per cent oxygen. Rates of oxygen consumption (QO_2) were measured over a wide range of increasing temperatures at about 1.5°C. intervals and are expressed as microliters of gas consumed per milligram initial wet weight of tissue per hour. Single tissue samples were used and the temperature was maintained constant until QO_2 was constant, usually about 10 minutes after thermoequilibration (also 10 minutes) with 1° to 2°C. increase in temperature. The same tissue sample was then run at a higher temperature and another determinaton of QO_2 made until the desired temperature range was covered.

RESULTS

Animals.—The absolute levels of metabolism of these animals vary because of many factors, including size of the animal and large changes in water

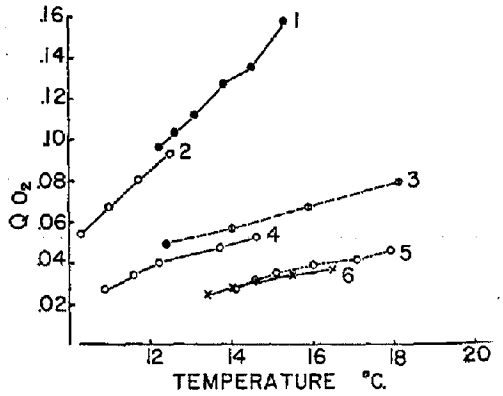

FIG. 1. The QO_2 of several poikilotherms at various temperatures. Each line represents a single animal studied at the temperatures indicated. Lines 1 and 2 are from *Hermissenda crassicornis*, line 3 from *Ensatina eschscholtzii*, and lines 4, 5, and 6 from *Leptasterias pusilla*.

content. The latter is particularly responsible, since QO_2 is here expressed on a wet weight basis. The absolute level is of little importance in the present study, however. It is clear that the QO_2 increases regularly with temperature and the QO_2-temperature curve is without peaks or dips. The QO_2-temperature relationship for the marine nudibranch *Hermissenda crassicornis* is shown in Fig. 1 (lines 1 and 2). Nine animals were studied (unusual Pacific storms prevented further work with this form) and results are shown for two typical specimens. The salamander *Ensatina eschscholtzii* (6 animals) was investigated and one example is shown in Fig. 1 (line 3). Fig. 1 also shows results for the starfish *Leptasterias pusilla*. Fourteen animals were studied; three are shown in the figure (lines 4, 5, and 6). As may be seen from the figure there is no evidence of peaks or dips in the relationship between metabolic rate and temperature.

Other animals were investigated over suitable temperature ranges and are listed in Table I. All these poikilotherms reacted similarly to increasing temperature and all experiments with each species showed regular curves when QO_2 was related to temperature.

TABLE I

Animals in Which QO_2 Was Determined at Very Small Temperature Intervals. QO_2 Always Increased Regularly with Increasing Temperature and the Resulting Curves Were without Peaks or Dips

Animal studied	Temperature range
	°C.
*Hermissenda crassicornis**	9.5–20.2
*Leptasterias pusilla**	10.5–18.1
*Ensatina eschscholtzii**	15.0–22.8
Mopalia muscosa	10.6–16.8
Anisodoris nobilis	10.0–15.1
Strongylocentrotus purpuratus	13.9–18.7
Petrolisthes cinctipes	10.4–18.7
Patiria miniata	11.0–16.5
Lumbricus terrestris	13.0–18.4

* QO_2-temperature curves shown in Fig. 1 for representative animals.

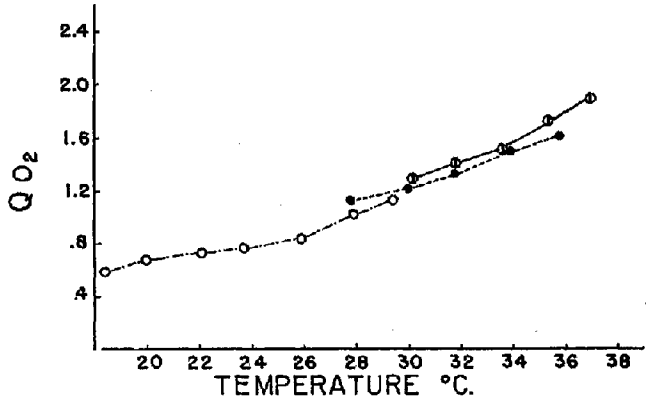

FIG. 2. The QO_2 of brain slices at various temperatures. Each line was obtained with a single brain sample from one rat.

Tissues.—Both skin and brain of rats, when properly prepared, have a constant QO_2 for 3 or 4 hours at 30° to 35°C. and much longer at lower temperatures. Thus it was possible to determine QO_2 at many temperatures on a single sample of skin or brain over a range of 8° or 10°. By studying overlapping ranges and many separate samples the entire relationship between

temperature and QO_2 becomes clear. All data were recorded on single samples so that any "peaks" in metabolic rate would not be obscured by averaging data. Fifteen samples of brain from 10 animals were used and representative curves are shown in Fig. 2. The range studied was from 17.9° to 40°C. and no dips or peaks were observed. Fifteen samples of skin from 15 animals

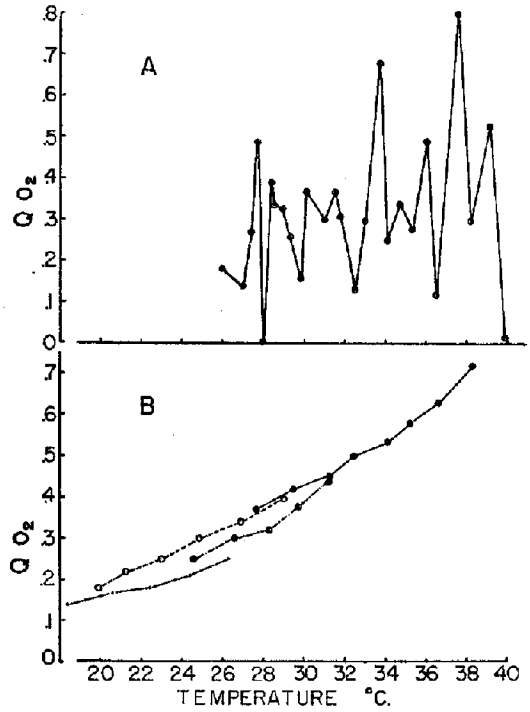

FIG. 3 A. The QO_2 of a single skin sample at various temperatures (respirometer experiment) as reported by J. M. O'Connor (redrawn from his Fig. 1 (reference 1957) which also shows similar lines for the QO_2 of skin using the Vierordt method).

FIG. 3 B. The QO_2 of four single samples of skin at various temperatures determined by the authors (respirometer experiments).

were studied over the range 18.4 to 38.3°C. and representative curves are shown in Fig. 3 B. Again the curves are regular.

For comparison O'Connor's results with rat skin (O'Connor, 1957) are also shown in Fig. 3 A. Since this is the relationship between temperature and metabolic rate which he states is the true one, and since he has challenged a long accepted fundamental rule of metabolic response, it is important to compare his results on skin with those reported here.

DISCUSSION

It would be impossible to list the hundreds of studies of the effects of temperature on oxygen consumption. The pertinent ones would, if they were to confirm or deny the presence of peaks of oxygen consumption with increasing temperature, be those in which single animals or samples were studied at very small temperature intervals, and in which there was no averaging of data, since O'Connor states that the peaks and dips may shift in position. We have found no such studies. Also, reference to well-known monographs on the biological effects of temperature revealed no data which do show dips or peaks in the metabolism-temperature curves (*cf.* Bělehrádek, 1935; Johnson, Eyring, and Polissar, 1954; Precht, Christophersen, and Hensel, 1955). It must be understood that the existence of a thermal optimum for these metabolic processes results in lower rates both above and below this optimum. This single high point in any metabolism-temperature curve is not at all comparable to O'Connor's multiple "peaks," nor are the changes in slope (called "breaks" by Crozier) of such curves. O'Connor found (1957) "Seven peaks followed by marked falls within the temperature range of 10° to 42°C." Studies in which small temperature intervals were examined do exist (*cf.* Krogh, 1916; Crozier, 1924; Crozier, Tang, and French, 1934) but investigators have either used groups of organisms (*Chlorella*, yeasts, *etc.*) or have used averaged data so that peaks of metabolism, if present, could be obscured.

Recently Rummel, Jacobi, and Pfleger (1956) reported in a preliminary communication that the QO_2 of guinea pig intestine *in vitro* was no higher at 37° than at 34°C; there was a plateau between those temperatures. After we had completed most of the experiments reported here, Rummel *et al.* (1957) published a complete report of their work in which they found QO_2 to increase with temperature from 27° to 37°C. in a regular way without a plateau. The plateau reported earlier was found to be a result of contraction of the intestine after placing it in the respirometer vessel so that at temperatures above 34°C. diffusion of oxygen became limiting; this difficulty was avoided by using thinner pieces of intestine from young animals.

O'Connor has suggested (1955, 1957) that his peaks are a result of alterations in the physical state of insoluble fatty acids in monolayer. The peaks are said to be associated with the seven naturally occurring saturated fatty acids from caproic to stearic. Oleic acid is also said to produce a peak which is close to or identical with that due to lauric acid. Since other investigators have not designed experiments to show the presence or absence of these peaks, we can only report that our data, which were obtained for this very reason, do not show them. We have used one tissue (rat skin) and one animal (earthworm) which O'Connor himself used and found no evidence of irregularities in the course of the metabolism-temperature curves. Further experiments

with brain slices, and with several other animals support this finding. Comparison of the two parts of Fig. 3 [our results for skin (B) shown with O'Connor's results for skin (A)] is especially informative. We can only conclude that our results lead one to take a skeptical view of a relationship between oxygen consumption and temperature which is not a regular and progressive one.

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