The regulation of metabolism at different temperatures in growing and adult *Locusta migratoria* L.

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KURZFASSUNG: **Die Regulation des Stoffwechsels bei verschiedenen Temperaturen durch wachsende und adulte *Locusta migratoria L.*** Es wird die Ansicht vertreten, daß Veränderungen der Umgebungstemperatur den Stoffwechsel poikilothermer Tiere beeinflussen durch den Mechanismus der Temperaturperzeption sowie durch zentralnervöse Integration, Hormonausschüttung und schließlich Hormonwirkung auf die Körperzellen. Daß eine hormonelle Regulation stattfindet, geht aus folgenden Experimenten hervor. Wenn man das Frontalganglion entfernt, hört das Körperwachstum auf und die Ovarien reifen nicht. Stichproben mit dem Elektrophorese-Verfahren ergaben, daß das Blut kein Protein enthält. Ähnliche Resultate erhält man nach Durchschneiden der posterioren Pharyngealnerven oder der Frontalkonnektive. Zytologische Untersuchungen am Neuroendokrinen-System operierter Wanderheuschrecken zeigen, daß eine Akkumulation neurosekretorischen Materials in den Nervi corpori cardiaci I stattfindet und daß das Corpus cardiacum abnormal ist. Experimente, in denen aus Fettkörper und Flugmuskeln isolierte Mitochondrien benutzt werden, ergaben, daß eine vermehrte Sauerstoffaufnahme eintritt, wenn den Präparaten frische Corpora allata zugesetzt werden. Um nun zu entscheiden, ob diese hormonale Kontrolle des Stoffwechsels die Reaktionen auf veränderte Umweltbedingungen tatsächlich beeinflußt, wurde der Stoffwechsel intakter Ganztiere gemessen, und zwar indem die Veränderungen der Körpertemperatur unter sorgfältig kontrollierten Bedingungen registriert und als Indikator der Stoffwechselrate interpretiert wurden. Der Vorteil dieser Methode liegt vor allem darin, daß Veränderungen fast augenblicklich nachgewiesen werden können. Nach einer Erhöhung der Umgebungstemperatur von 20° auf 40° C hinkte die Körpertemperatur zunächst hinterher; dann stieg sie gleich schnell mit der Umgebungstemperatur an und übertraf diese schließlich. Die resultierende Kurve ist komplexer, als man nach den Wärmediffusionstheorien annehmen sollte; sie läßt sich in Einklang bringen mit Veränderungen, wie man sie für eine neuroendokrin kontrollierte Reaktion postulieren würde. Der Kurvenverlauf konnte durch Injektionen mit 2:4 Dinitrophenol noch stärker zum Ausdruck gebracht und durch Injektionen mit subletalen Kaliumzyanid-Dosen abgeschwächt werden. Dieser Sachverhalt und ähnliche Ergebnisse, welche mit zwei anderen Apparaturen erhalten wurden, zeigen, daß metabolische Reaktionen eine wichtige Rolle beim Zustandekommen der erwähnten Kurve spielen.

INTRODUCTION

It is generally held that in poikilothermic animals the metabolic rate is closely related to the environmental temperature. The causal chain of events being, that a change in environmental temperature causes the cells of the animal to be passively heated or cooled, resulting in a speeding up or slowing down of the chemical reactions involved in cellular metabolism. The alternative view is that the animal's metabolism is at all times under the control of the creature's neuroendocrine system. The chain of events here is that a change in environmental temperature is sensed by the insect's temperature receptors, this information is fed to the central nervous system, then through the release of hormones a level of metabolism reached which is the most economical under the environmental conditions imposed.

This latter view is one which I have been interested in (CLARKE 1960), and although the evidence for it is still rather fragmentary, I should like to talk about some recent work done in my laboratory which supports it.

RESULTS

In some recent experiments (CLARKE & LANGLEY 1963) it was found that the growth of the locust could be stopped at any time during its development by removing the frontal ganglion. When this operation was performed on early 3rd instar locusts, they showed an initial loss of weight in the 24 hours immediately following the operation. The animals then started to feed and showed an increase in weight due to filling the gut with food. This brought their weight to within a few percent of what it was immediately before the operation. They remained constant at this weight until they died, still in the 3rd instar, some 300 hours later. The operated controls also showed a loss of weight immediately following the operation. Feeding commenced, however, in 24 hours, and the animals grew normally, approximately doubling their weight, and moulting to the 4th instar in about 160 hours. This represents a lag of about 24 hours behind normal unoperated animals.

The effect of cutting both frontal connectives was the same as that of removing the frontal ganglion. Cutting one frontal connective resulted in no detectable differences between the operated animals and the operated controls. The cutting of the recurrent nerve and the outer oesophageal nerve was without effect on the further growth and moulting of the insect.

Anatomical studies of the innervation of the pharynx by the frontal ganglion showed that branches of the posterior pharyngeal nerve arise from six stretch receptors, three lying on each side of the mid-line of the pharynx. The identification of these organs rests entirely on anatomical similarities to published drawings and photographs. No electrophysiological studies were undertaken.

By separating the frontal ganglion from the surface of the pharynx with a fine seeker it is possible to cut these nerves and thus isolate the frontal ganglion from the stretch receptors. This had the same effect as the removal of the frontal ganglion, the insect living and being freely active for some 200–300 hours after the operation, but failing to grow. This effect shows that the frontal ganglion does not produce a hormone.

An examination of the neurosecretory system of the brain and corpus cardiacum of operated insects and operated controls was carried out using Gomori's chrome haematoxylin and phloxine stain for the demonstration of neurosecretory material.

The operated animals differed from the controls in that neurosecretory material packed the nervi corpori cardiaci I, and that the corpus cardiacum was of very abnormal appearance.

These facts were interpreted as follows: movements of the pharynx (mostly concerned with the passage of food material) activate the stretch receptors. Information from these was passed to the brain via the posterior pharyngeal nerve, frontal ganglion and frontal connectives where it influenced the production and/or release of hormones from the corpus cardiacum. Surgical interference with the integrity of this system results in the nonrelease of hormones from the corpus cardiacum.

Neurosecretory material has been implicated by THOMSEN & MØLLER (1963) in the synthesis of proteinases in the blowfly, and in its absence the ovaries failed to mature. In the adult locust in which the frontal ganglion had been removed, there is also a failure of the ovaries to mature (CLARKE & LANGLEY 1963).

These observations suggest that the neurosecretory material may have a general effect on protein synthesis, if so, this provides an obvious biochemical basis for our observations. Preliminary electrophoretic studies showed an absence of protein from the blood of operated animals compared with its presence in operated controls. This aspect of protein metabolism will be dealt with more fully by Mr. C. Gillot who has continued the studies started by Dr. Langley and myself in this field.

Considerable attention has been given to the role of the corpus allatum hormone in the regulation of metabolism by many workers. It is difficult, however, to ascertain whether the metabolic changes observed are secondary to its action as a juvenile and gonadotropic hormone or a direct primary action on metabolic systems in all tissues. Some time ago I was able to make a few experiments on the effect of the corpora allata hormone on the oxygen consumption of mitochondria using a succinate substrate (CLARKE & BALDWIN 1960). The mitochondria were prepared from the flight muscle and fat body of the locust. Both samples showed an increase in oxygen consumption over control values upon the addition of ground fresh corpora allata.

These experiments show that the metabolism is controlled by hormones, but whether this control can override the effects of temperature or whether it simply adds fine adjustments to a temperature set rate requires further investigation.

One solution to this problem is to measure the metabolic rate of the whole animal under changing temperature conditions and to study the effect of the removal of the endocrine organs, or of interference with the sensory inflow of the animal, on the responses to be expected from normal animals. This work is in progress and presents numerous technical difficulties. I should like in the final part of this paper to discuss some of the problems associated with the measurement of total metabolic rate in insects particularly under changing temperature conditions.

The first problem is to try to get the insect to conform to a standard treatment prior to the measurement being made. The insect should be in the same stage of the moulting or reproductive cycle. Whilst simple inspection of the animal will give broad indications of age, accuracy is only possible by rearing the insect under carefully controlled conditions and knowing the age from the last moult. Variation between individuals is large and cannot be eliminated. It can be reduced by selecting at each ecdysis those individuals moulting within a limited period around the mean time of ecdysis. In practice this gives a reasonably uniform population and further selection by inbreeding appears unnecessary.

The best standardisation of food intake is obtained by supplying the animal with abundant food the whole time and allowing it to feed as often as it wants. In starved insects the weight and oxygen consumption decrease throughout the whole period of starvation, no level plateau is reached, and no constant value of oxygen consumption per milligram occurs (CLARKE 1957). The giving of a single meal to a starved animal results in a rapid increase in weight due to food intake; the oxygen consumption remains at starvation level for 4 hours after feeding and then shows a peak of some 5 hours total duration, so here again no level plateau on which comparative measurements could be made is attained. In fact insects do not show a 'post absorptive resting state' and basal metabolism as understood in vertebrate studies cannot be said to exist in insects.

The insects studied therefore are those grown under condition of least stress, their age is known within ± 6 hours, and weight measurements indicate that normal growth has taken place in the days prior to the measurement being made.

The physiology of insect respiration imposes limitations on the use of indirect methods of estimating metabolism. The periodic excretion of carbon dioxide means that observations have to be continued over about 6 hours if errors arising from the chance inclusion or exclusion of an outburst are not to seriously affect the results. Furthermore, these methods are temperature sensitive, hence observations at different temperatures have to be interspersed with periods for equilibration. The time thus taken to make a series of measurements allows both for changes in the animal due to natural development and to the exhausting character of the tests imposed.

More direct measurement of metabolism by heat production in a calorimeter is particularly difficult for small terrestrial animals. Water loss from the animal in dry air can cool the apparatus and falsify the results. It is practically impossible to maintain a water content of the air which will prevent water loss from the animal, or to determine the amount of this loss since weight changes in the animal are confounded with faecal loss. The time required for enough heat to be liberated from the insect to warm even a small calorimeter is long enough for starvation to influence the animal's metabolism.

Heat output is measured in a calorimeter as the product of the temperature rise, the mass and the specific heat of the calorimeter. If the insect's body is used as its own calorimeter and its temperature measured under carefully controlled conditions, the changes in temperature can be used as an indication of changes in metabolism. The specific heat of the insect's body can be regarded as constant for the duration of the experiment, the mass changes very little (1–2 %) during the course of the experiment, and in practice these changes can be ignored. The great advantages of this method are as follows: –

(a) Changes in body temperature can be quickly detected, hence changes in metabolism indicated by this measurement can be detected almost as soon as they occur.

(b) Environmental temperatures and humidities can be easily changed and there need be no period for equilibration before the effects of a temperature change can be measured.

(c) The time necessary to subject the animal to a spectrum of temperatures is short and the animal is not exhausted during the course of an experiment.

In the original apparatus warmed air was passed over sulphuric acid solutions to ensure a constant relative humidity; metal radiation screens were used to protect the animal from radiant heat. The air and body temperatures were measured independently using copper constantan thermocouples with the cold junction at 0° C. The potential developed was measured with a Cambridge Potentiometer (CLARKE 1960).

In a more recent apparatus air was first saturated at a given temperature in one water bath and then raised to another temperature in another water bath. The temperature in both water baths could be raised at an independent through constant rate, thus ensuring that a constant relative humidity was obtained at all temperatures. The animal was protected from radiant heat by a pair of perspex screens. The environmental temperature was measured by a pair of thermocouples, the cold junction being at 0° C. The difference between the body temperature of the locust and the environment was measured by an 8 junction thermopile. The potential from both sets of thermocouples was amplified and fed to a two pen millamp recorder. In this way continous changes of both body and air temperature could be recorded simultaneously. Both types of apparatus gave similar results and thus showed that the changes in body temperature did not depend upon some point in the geometry and nature of the apparatus. At a steady environmental temperature the body temperature of the locust comes to a steady value and remains constant for a period of time. The body temperature may be equal to, above or below that of the environment regardless of the actual value of the environmental temperature providing it is within the physiological range of the insect.

If the insect is equilibrated to an environmental temperature of 20° C and then the environmental temperature slowly raised to 40° C the following changes in body temperature occur. There is immediately an increase in body temperature which, however, is slower than that of the environment. When the body temperature has lagged 1–2° C behind the environmental temperature both then increase at the same rate. The magnitude of the lag is approximately the same regardless of the mass of the insect. As the rise in environmental temperature begins to slacken the body temperature continues to increase until when the environmental temperature is at its upper constant level the body temperature is 0.5–1.0° C above it. Physical objects of the same mass and similar in shape to the locust show strict conformity to Newton's Laws of Cooling. As the environmental temperature is raised the objects temperature lags further and further behind until the environmental temperature becomes steady when the objects's temperature approaches the environmental temperature in a manner giving an asymptotic curve.

The response of the body temperature of the locust can be exaggerated by injections of di-nitrophenol, and reduced by injections of sub-lethal doses of potassium cyanide (CLARKE 1960). This effect clearly shows that metabolic reactions play a large part in the production of the temperature curve described, and simple diffusion of heat into the animal a minor part.

To sum up, the experiments quoted in the first part of this paper indicate that the metabolism of the locust is under control of its neuroendocrine system. Those in the second part show a response to temperature change more complicated than heat diffusion theories require, but of a nature one would postulate if temperature perception and hormonal release were the paths through which environmental temperature influences the animal's metabolism. Reaching a little ahead from these conclusions it is tempting to suggest that Claude BERNARD's famous dictum might be modified and extended to 'a controlled response to the environment is the condition for a free life'.

SUMMARY

1. It is suggested that the locust's metabolism is at all times under the control of the neuroendocrine system and that metabolic response to a temperature change is mediated through temperature receptors, central nervous system, and hormonal release, followed by cellular metabolic response.
2. The mechanism of the control of post embryonic growth of the locust via pharyngeal stretch receptors, posterior, pharyngeal nerves, frontal ganglion, frontal connectives, and hormonal release from the corpus cardiacum is described.
3. In the absence of neurosecretory material being released it is postulated that there is a failure in protein synthesis.
4. Attention is called to the stimulating effect of the corpus allatum hormone on metabolism, as shown by an increase in the oxygen consumption of mitochondria prepared from locust muscle and fat body when corpora allata are added to the preparation.
5. To help to decide whether this hormonal control of metabolism influences the response the locust makes to changing environmental temperatures the metabolism of the animal must be measured under these conditions. The problems of measuring metabolism in whole animals are discussed.
6. It is suggested that the pre-experimental treatment most likely to reduce variation amongst the population is that of rearing the animal under controlled conditions in the presence of abundant food, and selecting from the population those of average growth rate.
7. The limitations of indirect methods of measuring metabolism for the problem in hand are discussed, as also are limitations of determining heat output by ordinary calorimetric methods.
8. A solution is suggested of using the insect's body as its own calorimeter and interpreting the changes of body temperature as reflecting changes in metabolism. The advantage of this method lies in the speed with which a response can be detected, and the ease with which environmental conditions can be kept either constant or programmed for any change.
9. The curve of body temperature obtained when environmental temperature is raised from 20–40^0 C shows features not seen in the non-living objects tested. This curve is more in accordance with the theory postulated, than with those depending upon simple heat diffusion into the animal.

10. The characteristics of this curve can be changed by injections of 2:4 dinitrophenol and sub-lethal doses of potassium cyanide, indicating that the raising of body temperature is largely due to metabolic reactions.
11. In conclusion it is suggested that Claude Bernard's dictum might be modified to 'a controlled response to the environment is a condition of free life'.

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Discussion following the paper by CLARKE

KRÜGER: Bei *Gryllus domesticus* findet sich bei der doppellogarithmischen Darstellung der Atmung als Funktion des Gewichts eine Unstetigkeit im 3. oder 4. Stadium. Der Wert für α bleibt bei 1, es erfolgt die Reduktion des Stoffwechsels durch Verringerung des Wertes für b. Eine (unveröffentlichte) Beobachtung an *Locusta migratoria* zeigte, daß diese Reduktion innerhalb von 24 Stunden erfolgt.

CLARKE: In our curves for the changes in weight and oxygen consumption for growth of locusts, the weight follows an exponential function terminating abruptly when sexual maturity is reached. The oxygen consumption follows approximately a more sigmoid curve, the change at the upper limit being less abrupt. The time at which this change occurs is variable (usually during the fifth instar). Thus in the late fifth and early adult instars, oxygen consumption per individual is fairly constant (under the conditions of the experiment), but the weight approximately doubles. Hence, oxygen consumption per milligram decreases. I think in this case there is a change in slope rather than in the 'a' factor of the regression equation $y = a + bx$. This is expressed by calculating two straight regression lines fort the oxygen consumption of the fifth instar. The first for early in the instar is always with a higher 'b' but lower 'a', the second with a higher 'a', but lower 'b' value. There may be a definite reason for this change. Dr. STAAL has pointed out to me that in his work on the phase changes in *Locusta,* he found that the growth of the corpora allata, which occurred throughout early development, ceased during the fifth instar at a time which roughly corresponded with the times I found a change in oxygen consumption.

VON BERTALANFFY: It may be mentioned that weight-proportionality of metabolic rate in insects was also found in our laboratory and by other investigators, both in hemimetabola and holometabola (e. g. *Dixippus:* MÜLLER 1943. *Z. vgl. Physiol.* **30**, 139–144; *Tenebrio:* VON BERTALANFFY & MÜLLER 1943. *Riv. di Biologia* **35**, 48–95). There is, however, a discrepancy between German and American investigators: Some of the latter claim that $M = bw^{2/3}$ is characteristic for hemimetabola, $M = bw^1$ for holometabola (G. A. EDWARDS 1953. *In:* K. D. ROEDER [ed.], Insect Physiology. Wiley, New York, pp. 96–146). These discrepancies should be ironed out. With respect to protein synthesis, I would suggest that RNA content be investigated as measure of its intensity in different cells. (This suggestion was answered by the subsequent presentation of GILLOTT, reporting RNA study with the methyl green-pyronine stain.) For this purpose the acridine orange fluorescence technique would appear the method of choice (e. g. VON BERTALANFFY 1963. *Protoplasma* **57**, 51–83).

ROBERTS: Have you tried using the drug dinitro-phenol on both operated and normal animals? Perhaps if respiration of the two experimental groups were found to be similar immediately, this too would suggest that the frontal ganglion effect is not endocrine.

CLARKE: We have not tried the effect of dinitrophenol injections on operated animals; it would certainly be worth trying. In normal animals the stimulatory effect of dinitrophenol was not observed when the animal was equilibrated at 20° C, and did not appear until the temperature was being raised. We observed the effect of dinitrophenol on the respiration of mitochondrial prepared from normal animals and found inhibition except in the presence of corpus allatum extracts. Unfortunately, since the effect of the removal of the frontal ganglion was discovered, it has not been possible to have access to the equipment necessary for the isolation of mitochondria. I do not think the frontal ganglion has an endocrine function in the locust since neurosecretory cells were not observed in it, and its presence in the body after the pharyngeal nerves had been cut, caused no observable difference from responses of animals from which it had been completely removed. However, in some insects, neurosecretory cells have been observed in the frontal ganglion, so a hormonal function for this organ in some insects should not be considered impossible.

SMITH: The crossover point at which body temperature rises above environmental may possibly represent the shift from heat loss by radiation plus conductance and evaporation to one in which only the latter two are effective.

CLARKE: I have not been able to measure separately the heat exchanges that occur due to radiation, conductance and evaporation. Conductance should remain constant throughout the experiment; the locust's physical contact with the perspex platform was small and unchanged during the course of the experiment. Evaporation, which might well increase with increasing temperature, would lower, rather than raise the body temperature. However, the saturation deficiency was kept constant, and the range of temperature used was below that necessary to dis-orientate the wax waterproof covering of the insect. Losses by radiant heat have not been measured, but the fact that the body temperature can be altered by metabolic agents suggests that losses by radiant heat, which would depend upon the absolute temperature and would presumably be nearly constant in all experiments, do not substantially enter into the picture.