CALORIMETRICAL EXPERIMENTS ON WARM-BLOODED ANIMALS.

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VARIOUS types of calorimeter have been designed for investigating the total energy exchanges of small warm-blooded animals, of which instruments a good description has been given by Rubner[1]. Among them may be mentioned Lavoisier and Laplace's ice calorimeter, Lavoisier and Crawford's water calorimeter, the air calorimeters of Geigel, d'Arsonval, Richet, and Haldane, and the respiration calorimeters of Rubner and of Attwater and Benedict. Most of these are complicated to work, and some of them are not very accurate. Now in all "nutrition work" it is advisable, whenever possible, to make the results statistical in nature, by reason of the very great variability of the material used. Experiments which are complicated and difficult to perform can seldom be carried out on a scale sufficiently extended to satisfy the doubts of the statistician: it is therefore desirable in experiments performed upon the total evolution of heat by animals, to avoid excessive labour by using a calorimeter which shall work automatically over long periods, with some considerable degree of accuracy and simplicity. It occurred to us that the main difficulty of such experiments, viz. loss of heat by conduction and radiation, might be avoided by the use of large Dewar flasks as heat-insulators in which to keep the animals during the experimental periods. Adopting their use, the first problem that arises is how to collect and estimate the heat given out by the animals—the Dewar flask is so perfect a non-conductor that unless some measures are adopted for taking away the heat formed it will practically all remain in the flask, and in a short time the animals will die. The natural suggestion was that the animals should be kept cool by a stream of air passing into and out of the flask, that the rise of

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[1] *Tigerstedt's Hdb. d. physiol. Methodik.* I. "Biokalorimetrie."

temperature of the air passing through the flask should be measured by a thermo-electric arrangement, and that this rise of temperature multiplied by the rate of flow of the air and an appropriate constant would give an exact measure of the heat-production of the animals. Simple quantitative considerations however showed this idea to be absolutely impossible: a rat of 100 gms. gives out approximately 20,000 calories per day[1], say 1000 calories per hour, say 0·2 calories per second. It requires only 6·8 cal. to warm 22·2 litres of air 1° C.: supposing the outgoing air were 4° C. warmer than the ingoing air, this allows us to carry away 27 cals. with 22 litres, or about 1 cal. per litre: hence even for a rat of 100 gms. we should have to force in about 200 c.c. of air per sec.: such a quantity would be very difficult to regulate in a 3 or 4 litre flask, and moreover the "draught" would be very unpleasant for the animal. For short periods of course an animal can keep itself cool by evaporation, and the heat it gives out can be measured by the extent of this evaporation. Such a method is, however, quite out of the question in long experiments. Another, and the final suggestion, was to circulate water inside the flask in a metal tube, so as to take away the heat liberated, and to measure the heat liberated by means of a thermopile or thermocouple registering the rise of temperature of this water. The rate of flow of the water in c.c. per minute, multiplied by the difference of temperature in °C. between the ingoing and the outgoing water, would give in gram-calories per minute the rate of heat production of the animal. Several little mechanical difficulties were found in the development of the idea, but after these were overcome the method was found to work perfectly. In the stage which the apparatus has now reached all the manipulation required is:

(1) to turn on two taps, one to provide air, and the other the water for collecting and measuring the heat:

(2) to measure the (absolutely constant) rate of flow of the water through the coil of tubing inside the flask:

(3) to place the animals in a zinc cage inside the calorimeter:

(4) to connect up a self-recording galvanometer: and

(5) to find the area of the curve marked out by the galvanometer, and to multiply this by the rate of flow of the water.

The number so obtained, multiplied by an appropriate constant, is the total heat produced by the animals; thus with, at the most, 30 mins. labour one may obtain, correct to about 2 %, the total heat

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[1] Throughout this paper calorie means gram-calorie.

given out in 24 hours by one or more rats, mice or other small animals, up to the size of a guinea-pig. The experiment may moreover be continued for any period desired even to several days, with no more trouble than that of changing the paper in the recording galvanometer every 24 hours.

The body of the calorimeter has consisted, in every case, of a cylindrical, silvered, double-walled vacuum flask[1]. Altogether four calorimeters have been constructed, two of 8 cms. internal diameter and some 40 to 45 cms. depth, one of 14 cms. diameter and 50 cms. depth, and one of 16 cms. diameter and 60 cms. depth. The smaller ones have been used for mice or individual small rats: the larger ones for one or more large rats, or other animals. Unfortunately the largest flask broke before it had been long in use. It was too large to bear safely, when lying in a horizontal position, the weight both of tubing and animal. The flask of 14 cms. diameter has however been in use for six months. In constructing the calorimeter the flask is laid horizontally in a box filled with sawdust, with its open end sticking out one or two cm. through a hole in the wood, and is there fixed by stuffing in cotton-wool tightly around its neck. The sawdust and cotton-wool serve both to save it from shocks, and also to shield it from temperature-changes outside. Such shielding is of some importance, for the vacuum between the walls is never so complete that one may afford to expose the outside wall to considerable changes of temperature. In order to avoid errors due to conduction through the walls and across the "vacuum," we have adopted the further device of passing the coil of tubing conveying the water around the outside of the flask, before passing it around the inside. It is always possible that this water, which flows from a tank, through a copper- or lead-coil inside the flask, may be 2 or 3° C. cooler than the room: in this case if the outer wall were allowed to be at the unknown and variable temperature of the room and the inner at the temperature of the water-coil with which it is in contact, there might be a considerable and incalculable error due to the loss of heat through the "vacuum." Seeing however that the water, before passing into the flask, flows through a coil of 20 to 40 turns of thin lead-tubing coiled around the outside of the flask, it follows that the outside is preserved very nearly at the temperature of the inside[2], so that loss of heat by conduction through the vacuum must be small.

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[1] The smaller by Townson and Mercer, the larger by Burger of Berlin.

[2] The rise of temperature of the water, on its journey through the coil inside the flask, is rarely more than 1° C.

Moreover, any heat loss which still occurs is now calculable; such heat loss must be proportional to the average difference of temperature between the inside and the outside walls: the average inside temperature is the mean of the temperatures of the ingoing and outgoing waters, $\frac{T_1+T_2}{2}$ say: the outside temperature is T_1, viz. that of the ingoing water: the difference between these, viz. $\frac{T_1+T_2}{2}-T_1=\frac{T_2-T_1}{2}$, is half the quantity observed at every moment with the thermopile: thus the heat-loss is calculable, being simply the product of $\frac{1}{2}(T_2-T_1)$ and a coefficient of heat-loss which can be determined by experiment.

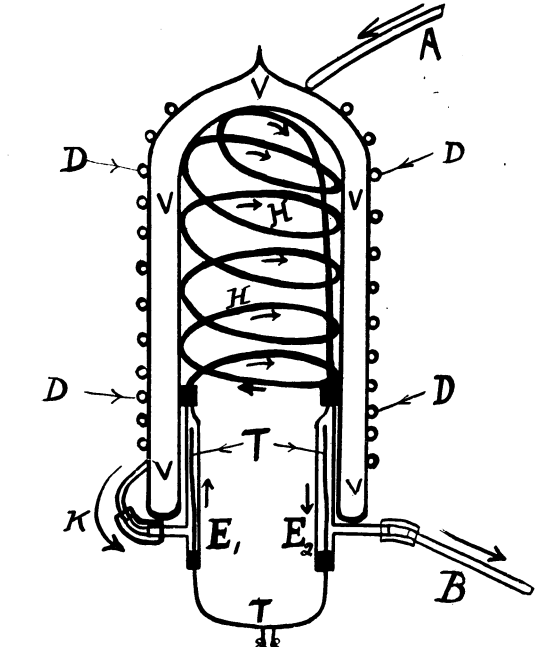

Fig. 1. Horizontal section of calorimeter. *V, V, V, V, V* vacuum between walls of cylindrical Dewar flask. *A* incoming water, from tank. *D, D, D, D* sections of lead tubing wound around the outside of the flask. *K* junction between outer lead tubing and T-piece E_1. E_1 and E_2 inlet and exit T-pieces containing *T* the thermopile. *H, H* coil of lead tubing inside the flask. *B* exit pipe for water to fall into a sink. *G* self-registering galvanometer ("thread recorder").

The tubing used to form the inside and the outside coils should be of lead (composition), of inside diameter 3 or 4 mm. Copper tubing was originally tried, as being a better conductor of heat, and is still in use in one of the smaller flasks: there was found however to be no particular advantage in its use, and it is not easy to bend it to the required shape. In winding the inside coil of tubing a cylinder is first prepared of perforated zinc and of the appropriate size, say two-thirds the length of the flask and 10 mm. less in diameter. On this the lead tubing is wound tightly and the whole is then inserted right up as far as it will go into the flask, which it should fit exactly. To hold the animals another cylindrical zinc cage is made, of approximately the same size, only a shade smaller: it can thus be slipped in and out of the first zinc cylinder, which remains fixed inside the flask. The outer tubing (*D*, *D*, *D*, *D*, Fig. 1) is wound directly on to the outside of the flask: one extremity of it, *A*, passes to the water reservoir, and the other, *K*, is connected to the inside coil, *H*, by means of a glass T-piece, *E*, which also holds one end of the thermopile, *T*. The other extremity of the inside coil is connected to another T-piece, E_2, which holds the other end of the thermopile, and through which the water passes away to the sink, or to the measuring cylinder.

In order to obtain water at an approximately uniform temperature to circulate in the coils we have erected a large galvanized-iron tank, into which water is continually running from above, and keeping at a constant level by means of an overflow pipe. Water is taken from the bottom of this tank, and found to maintain a very uniform temperature over long periods. The tank is much higher than the flasks, so that a head of water of some 180 cms. is available for forcing water around the coils. The tubes which lead the water down from the tank to the coils, and from the coils to the sink should be narrow, and preferably of lead, not of broad glass or metal tubing. This is of great importance; in wide tubes gas-bubbles will collect, of varying size, the water running round the edges of them. This leads to a large fall of the pressure available for forcing the water along, and may cause variations as large as 50 % in the flow. Such variations of course render the whole method perfectly hopeless, for the latter is based on the constancy of the water flow. By using narrow lead or glass and rubber tubing we have often secured a constancy of the water flow to within about 0·1 % over periods of 24 hours, and always a constancy to within about 1 %. This is all that can be desired, so that absolute confidence may be placed in the constancy of the water flow, and readings made merely at the beginning and the end of an experiment. The rate of flow can be varied by varying the head of pressure available: this is done simply by raising or lowering the end *B* of the rubber and glass tube from which the water, having traversed the whole system and passed through the second T-piece and over the warm junctions of the thermopile, falls finally into the sink.

As described above, the coil of tubing D wound around the outside of the flask is connected at the end A by a short piece of rubber with a clamp on it, to the lead pipe bringing down the water from the tank. At the other end K it is connected to the coil of tubing H inside the flask, which coil in its turn is brought back and connected through the T-piece E_2 to the pipes B taking the water to the sink. The T-pieces E_1 and E_2 are necessary for the introduction of the two ends of the thermopile into the stream of water, which runs around them, the outer ends of the T-pieces being blocked up to prevent the water from escaping. The inner ends of the thermopile, where the rise of temperature of the water is measured, are some 8 to 10 cm. inside the flask.

The construction of this thermopile, its insertion into the stream of water, and the blocking of the ends A provided more difficulty than all the rest of the construction together. For the preliminary experiments a sensitive mirror-galvanometer was used to record the current obtained from the constantan-copper junctions: and with this, a single thermocouple provided ample E.M.F. to carry out the experiments. This was made in two or three minutes with fine copper and coarse constantan wire, soldered at their ends and shellaced. It took up so little space that a T-piece of 4 mm. inside diameter could be used, and after insertion of the thermocouple, its ends filled up with plasticine. This was very simple and satisfactory, and all the preliminary experiments were made with it. It was obvious however that if the calorimeter was to be worth anything in practice it had to be self-recording. A continuous record of the current through the galvanometer might, no doubt, have been made photographically. This would, however, have been very cumbrous and expensive, and the course of the experiment would never have been visible till after the development of the paper or film. The idea of photographic registering was therefore discarded, and after preliminary experiments had rendered it certain that the method would work, a "thread recorder," an automatic registering d'Arsonval, was obtained by Dr F. G. Hopkins for these experiments and others, from the Cambridge Scientific Instrument Co. by means of a grant from the Royal Society. This thread-recorder makes it possible to take automatically a reading of the current from the thermopile either every half minute for 12 hours or every minute for 24 hours, after which another sheet of paper may be inserted, and the record continued. The water flow may be started, the recorder connected and wound up, and the whole apparatus left till the end of 24 hours, when the temperature record is found on the paper. The record also, at any time of the previous 10 hours, is always visible, which is a great convenience: and the rate of heat production at every moment can be calculated. There is, however, one disadvantage to its use: it necessitates the use of a thermopile of four to six pairs of junctions, instead of a simple thermocouple. With the most sensitive form of "thread recorder," that in which the suspension of the moving coil is a quartz-fibre, a constantan-copper thermocouple provides a deflection of about 16 mm. per 1° C. It is undesirable, as preliminary experiments showed, to warm the water inside the flask more than 1° C. In the first place larger differences of temperature between the inside and the outside faces of the flask cause errors due to conduction and radiation: the differential arrangement eliminates these largely but certainly not completely. Secondly, it is desirable to have a fairly rapid water flow in order to secure constancy thereof: and finally, for reasons given below, it is simpler that the air supplied for the animals to breathe should not have its temperature noticeably raised in passing through the calorimeter. It is therefore desirable not to have a temperature difference of much more than 1° C. between the ingoing and the outgoing water. The possible error in reading the record of the thread recorder is about one-half to one-fourth of a mm.: 0·5 mm. in 16 mm. is about 3 %, and a possible 3 % error is not good enough. For the preliminary experiments with the mirror galvanometer we knew that an accuracy to within about 1 or 2 % could be obtained with the whole process: to destroy this accuracy by a possible error of 3 % in one stage alone, would have been a pity and could be avoided. It was necessary however to use a thermopile of several junctions: for with a thermopile of say six junctions[1] there would be a sensitivity of 1° C. = 80 to 90 mm. and reading to 0·5 mm. this would mean an accuracy to within about 0·5 %, which is quite sufficient. But with the construction of this thermopile began the only real difficulties experienced. In the first place the thermopile had to be fairly small, in order to get into the T-pieces (the use of very large T-pieces being impossible). Its separate wires had therefore to be bound closely together. This led to short-circuiting between the wires, which being immersed in the water had their insulation of shellac destroyed. By

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[1] The electrical resistance of the thermocouple of six junctions finally constructed was 5 ohms, which is less than the resistance of the galvanometer.

the use of the following method however the difficulty was finally overcome. Six separate thermocouples as shown at *A*, in Fig. 2 were made of silk-covered constantan and copper wire. These were shellaced, and wound over from top to bottom with silk, and finally shellaced again and baked. The six were then bound together and shellaced, and their copper terminals soldered together in series as in the figure at *B*. After dipping the thermopile several times in hot melted paraffin-wax it was ready for use. The paraffin was used to perfect the insulation from the water. Another difficulty was experienced in fixing the thermopile fast in the T-pieces at the ends of E_1, E_2 (Fig. 1). In the first place, owing to the fact that the thermopile was made up of 12 separate wires, it was difficult to block up every separate hole between the wires, so that the water usually escaped between them.

Moreover, plasticine no longer held the water back, as it did previously with the single couple, owing to the much larger cross-section of the end *A* of the T-piece. Many attempts were made to fill up the ends with hot beeswax or paraffin: these however all failed, because the wax contracted on cooling and left spaces for the water to escape. Finally however these difficulties were overcome by the use of rings of india-rubber tubing. The thermopile was first dipped several times into paraffin-wax, until it had on it a fairly thick coating which filled up all the crevices between the wires. Over this paraffin were slipped rings of thick rubber, and the whole was then forced into the ends of the T-pieces. The rubber and paraffin together hold the thermopile

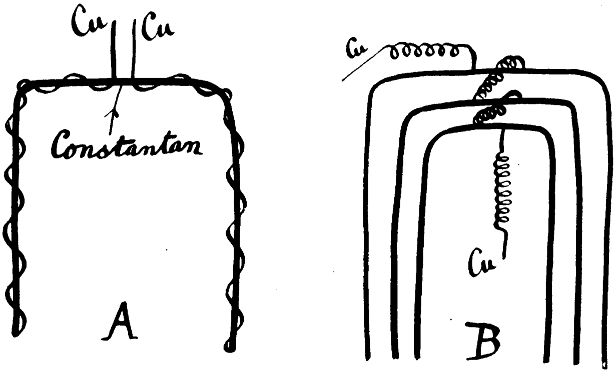

Fig. 2. Method of constructing the thermopile. *A* simple thermocouple, of copper and constantan. *B* three thermocouples in series, insulated with shellac and silk. These are then further tied together, and insulated with shellac and paraffin-wax. The actual thermopiles used each contained six thermocouples in series, and not three.

quite fast and water-tight. The two copper terminals of the thermopile are fixed to two brass terminals, stuck into the wooden box holding the flask: the wires to the galvanometer can be screwed down to these.

The degree of insulation obtained by this method of fixing up the thermopile is remarkable. The thermopile used in the large (14 cm. diameter) flask was of resistance

(*a*) in July, 1912, before putting it in the water ...	6·107 ohms
(*b*) in August, after putting it in the water ...	6·105 ,,
(*c*) in September	6·101 ,,
(*d*) in October	6·094 ,,
(*e*) in January, 1913	6·098 ,,

The thermopile of the smaller flask which has been used in these experiments had a resistance

(*a*) in October, 1912, of 5·005 ohms,
and (*b*) in January, 1913, of 4·998 ohms.

This degree of constancy is absolutely sufficient: we may therefore regard the insulation as practically complete, even if the thermopile remains immersed in water for five months.

The T-pieces containing the thermopile have been held firmly in the flask by the aid of plasticine.

The open end of the Dewar flask has been stoppered up by a large cork, containing two glass tubes to allow the air, necessary for the animals to breathe, to pass in and out. The cork has been held in position and been rendered completely air-tight around its edges by the use of plasticine. Of the two glass tubes, the one which allows the entrance of the air passes right up the calorimeter to the innermost end: the other, which takes away the air, terminates just inside the cork. By this means a flow of air is kept up along the whole length of the flask, and the greatest economy in the air supply is obtained. The air itself is sucked through the flask by a filter pump attached to a tap in the sink: if this pump fails to carry through any air when the inlet tube through the cork is closed it shows that the apparatus is completely air-tight. In the experiments given in this paper one condition has always been observed: the air is sent in saturated with moisture. This greatly simplifies the estimation of the heat-production, for evaporation of water in the flask is now impossible. If the animals evaporate in their lungs, the expired air is cooled again in the flask, and the contained moisture is deposited as water on the walls and pipes. Any heat therefore which has been lost in evaporation is regained in condensation. The air is not appreciably warmed (more than about one or two degrees centigrade) in its passage through the flask, and therefore cannot contain much more moisture on going out than on going in.

This avoids the estimation of ingoing and outgoing water-vapour, which would add to the labour of using the apparatus. The air, which comes into the flask from the room, is sucked first through three bottles containing water. By this means it is saturated with moisture.

It should be noted that this method of conducting the air supply is not essential, and in very exact experiments would be inadvisable for, as will be seen below, it is liable to a certain degree of error. The amount of the air supply must be regulated by the fact that it is not advisable to allow the CO_2 in the air breathed by the animals to reach a value of more than 1 $^0/_0$: 22 litres of CO_2 come from the oxidation of 30 grms. of carbohydrate. A 100 grm. rat gives out approximately 20,000 cals. per day when fasting, which is equivalent to about 5 grms. of carbohydrate, *i.e.* to 3·6 litres of CO_2. It is necessary therefore to supply the animal with at least 360 litres of air per day, in order to keep the CO_2 down to 1 $^0/_0$. Thus if we supply the animal with 500 litres per day we shall be safely within the limit. Now it is possible that these 500 litres may at times be warmed as much as 3° C. in passing through the flask: taking this outside limit the air may take away $\frac{1500 \times 6{\cdot}8}{22}$ calories, *i.e.* 464 cals. per day. Thus even if the greatest possible error occurred it would be not much more than 2 $^0/_0$ of the total quantity estimated. Direct control experiments however have shown that it is not so great as this, and in fact have failed to detect it at all. It has therefore been assumed that the loss of heat in the air current is negligible. Another possibility of error is that the air coming in is not completely saturated with water. Let us suppose that the air is only 95 $^0/_0$ saturated. The amount of moisture in 500 litres of saturated air at 15° C. is 6·36 grms.: 5 $^0/_0$ of this is 0·318 grm. The amount of heat which is absorbed on the evaporation of 1 grm. of water is about 580 cals.: the evaporation of 0·318 grm. would absorb therefore 185 cals., *i.e.* about 1 $^0/_0$ of the total heat liberated. It is unlikely again either that the air, if unsaturated when it went in, would be completely saturated in going out, or that the air is less than 95 $^0/_0$ saturated on going in. Thus again the error is probably smaller than the calculated 2 $^0/_0$. Finally it may be urged that, even if saturated air is provided the outgoing air is slightly warmer than the ingoing, and so is capable of taking up more water. In point of fact 500 litres will take up 6·75 grms. at 16° C., and 5·62 grms. at 13° C., a difference of 1·13 grms. This might lead to a loss of something like 656 cals., *i.e.* about 3 $^0/_0$ of the whole quantity estimated. There is thus, in the simple method of air supply adopted, the possibility of errors amounting to as much as 6 $^0/_0$ of the quantity measured. The actual error is probably considerably less, for every one of these calculations is based upon extreme cases. Anyhow, the error must not be laid to the charge of the calorimeter, for it would be avoided at once by measuring the water given out as vapour by the animals, and by ensuring that the air did not come out much hotter than it went in. In the present experiments however this has not been done, partly because it is not necessary to have extreme accuracy for them, and partly because they are all comparative, and therefore the errors are much the same in all. Of the experiments given here however the air supply is certainly the weakest point, and has led to the greatest possibility of error.

One virtue of this type of air supply is the fact that without any serious changes simultaneous observations can be made of the CO_2 output of the animals: all that it is necessary to do is to put two sulphuric acid bottles and two soda lime bottles in the order named in the path of the outgoing air, the increase in weight of the last two accounting for the CO_2 given out.

Let us turn then finally to the registering apparatus. As pointed out above, we can trust absolutely to the constancy of the rate of flow of water through the coil inside the calorimeter. For example, the following results are typical:

(1)	Time	10.46 a.m.	2.45 p.m.	8.15 p.m.
	Rate of flow, c.c. per min.	46·15	45·7	45·8
	Time	9.30 a.m.	1.30 p.m.	4.7 p.m..
	Rate of flow, c.c. per min.	45·44	45·8	46·2
	Time	10 a.m.	2.20 p.m.	10 a.m.
	Rate of flow, c.c. per min.	46·2	45·1	46·0
(2)	Time	10 a.m.	1 p.m.	5 p.m.
	Rate of flow, c.c. per min.	53·1	53·4	53·3

It is only necessary therefore to obtain a continuous record of the rise of temperature of this water. By means of the thread recorder an observation can be made every minute or half minute as desired. A typical curve is shown in Fig. 3. Before the experiment and after, the zero of the galvanometer is taken by disconnecting the latter from the thermopile. The line at the bottom of the paper represents this zero. The vertical height of the curve above this line then represents at every moment the difference of temperature between the ingoing and the outgoing water. The curve, as is seen, is usually a very irregular one; this is due to the animals moving about irregularly, eating, fighting with one another (if there is more than one animal inside), or going to sleep. In order therefore to obtain the total heat-evolution during any period it is necessary to find the average difference of temperature during that period. This is best done by finding the area of the curve in sq. mm. and dividing it by its total length in mm. The result is

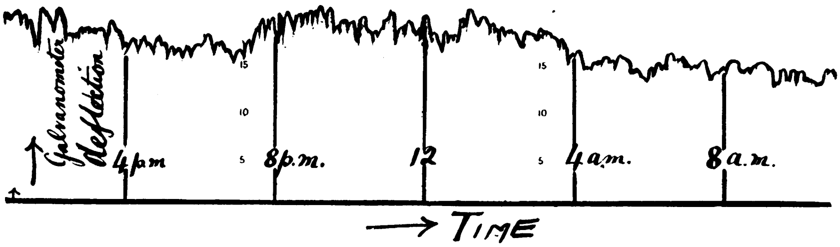

Fig. 3. Typical curve of galvanometer tracing, giving the rise of temperature of the circulating water at every minute for 24 hours.

the average vertical height in mm. during the period considered. This height has then to be interpreted in °C., which is done by the calibration method described below. The area of the curve has been found by weighing it: the weight being m, and that of the whole sheet being M, the area is m/M 34,000 sq. mm., the area of the whole sheet being 34,000 sq. mm. It can be better found by the aid of a planimeter which we have now obtained. The calibration method is as follows. A current of known fixed E.M.F., measured very accurately with a voltmeter, is passed through a resistance, also measured very accurately. The resistance is made of thick silk-covered constantan wire (being of constantan wire its value is almost completely independent of its temperature) and joined to thick flexible insulated copper wire. It is either inserted into a bottle of distilled water (through which the current cannot appreciably short-circuit), or else wound on a glass tube and insulated with paraffin and shellac, in order to give it a larger area wherefrom to conduct away heat to the coil of water tubing. The resistance and the size of the bottle are so chosen that with 4 volts applied the bottle's temperature finally reaches about 38° C. so that the system represents very well the exact conditions under which the experiments are carried out, viz. animals at about 38° C. in the flask. Any small errors which arise in one case will arise also in the other, and so will be controlled. One resistance we have often used has been 3·978 ohms, of which the leads (outside the calorimeter) represented ·03 ohm. The E.M.F. being 4 volts this would lead to a production of heat of

$$\left(\frac{4}{3\cdot978}\right)^2 \frac{3\cdot948}{4\cdot16} \times 60 = 57\cdot6.$$

calories per minute, or 82,900 calories per day, which is approximately the heat given out by 150 grms. of mice. Various other resistances also have been used. A very constant current can be obtained from two large 2 volt accumulators. The current is passed through the coil until a constant reading is obtained on the thread recorder. In this case the reading in mm. multiplied by the observed flow of water in c.c. per minute, is equated to the heat-production (*e.g.*, 57·6 cals.) calculated from the E.M.F. and resistance. The result gives the value of 1 mm. on the scale of the recorder in °C., after all corrections for heat-loss have been automatically made. In the case of the smaller flask seven of the latest determinations of the value of 1 mm. galvanometer deflection in °C. were, successively, ·0143, ·0133, ·0145, ·0145, ·0142, ·0140, ·0138. The mean value of these is ·0141° C., and the average error is only about 2·2 °/o. With the larger flask eight determinations were ·01685, ·01655, ·0167, ·0163, ·0167, ·0169, ·0168, ·0162. The mean value is ·0166°C., and the average error amounts to only 1·3 °/o. These experiments were all made under very much the same conditions as when a live animal is in the calorimeter: the flasks were stoppered up and made air-tight, and in some cases the air supply was passing. (It was found, however, that the latter had absolutely no influence on the result: see below.) Apparently, therefore, neglecting the errors due to evaporation described above, the method is capable of obtaining results on the average, correct to within 2 °/o. The virtue of the particular method sketched here of calibrating the thermopile, galvanometer and flask together by a direct experiment, is that the calibration takes place under the very conditions of the experiment; thus errors which arise every time are controlled and eliminated. For example, (*a*) the heat capacity of 1 c.c. of water at 20°C. is not quite 1: (*b*) there is a heat-loss through the walls of the flask, as described above: (*c*) the thermopile in the stream of water may not be absolutely at the temperature of the stream, because of conduction outwards, so that if calibrated by measuring the E.M.F. per 1°C. of the thermopile there might be a slight difference of reading. All such errors as these are avoided by the use of this method of calibrating under experimental conditions. Further, the apparatus can be calibrated whenever desired without taking it to pieces, which is a considerable advantage. Several experiments have been performed, both with the galvanometer and the "thread recorder," in order to verify the accuracy of the relation deduced above, viz. that the amount of air necessary to keep the animals normal will not take away any appreciable quantity of heat and therefore that the air supply need not lead to any appreciable errors. In the calibration experiments given below the value of 1 mm. on the recorder scale in °C was found to be quite independent of whether air was passing or not. (Exps. 3 and 4 are earlier ones, 3 with another thermopile, and 4 with a mirror galvanometer.)

Number of experiment	1	2	3	4
Air passing, 1 mm. =	·01499° C.	·01447° C.	·0129° C.	·000638° C.
Air not passing, 1 mm. =	·01497° C.	·01441° C.	·0129° C.	·000635° C.

Provided therefore that the air supply is not unreasonably large there will be no appreciable error due to the entire neglect of the heat carried away by the air itself. Of course this has no influence on any error due to evaporation, which in the calibration experiments could not take place at all, and can only be allowed for directly by estimating the degree of the evaporation.

It would be possible, by replacing the filter pump with a closed pump, and by circulating the air round and round, to estimate the oxygen used as well as the CO_2 produced: for the whole system would then be completely closed and the loss of oxygen could be measured. The filter pump is not satisfactory for this, as the water may take up or give out oxygen. As yet, however, we have had no time to design any simple completely closed pump, to circulate the air, so that simultaneous observations of the oxygen used have not been made.

In order to get absolute results in the measurement of the heat-production it is necessary to estimate very accurately in absolute units the currents and resistances used in calibrating the calorimeter. With the resistances, which being of constantan are the same when hot as when cool, there is no difficulty. The E.M.F.s have always been measured with a voltmeter—a microammeter by Paul with resistances from 50 to 100,000 ohms—which has been very carefully calibrated for the purpose with a cadmium cell. Very accurate observations of the E.M.F. are necessary, for its value occurs as a square in the expression for the liberation of heat, so that any error of observation is doubled.

EXPERIMENTAL RESULTS.

The experiments were directed towards elucidating two points: (*a*) the relation between heat-production and body-weight (or body-surface), and (*b*) the somewhat curious fact described by Hopkins[1] that two or more young animals grow faster when together than when kept separately. Incidentally the effects of food, in increasing the metabolism of rats kept at 15° C., has been found. This fact, accepting Rubner's results on the "specific dynamic energies" of foodstuffs, seems to confirm the results of (*a*), viz. that in animals of one species the total metabolism is not determined solely by the body area.

In the first place it should be noted that in all these experiments, which have extended over 5 months, the conditions have been kept very uniform. The temperature has seldom varied more than 1·5° C. from the mean, and all the observations have been made upon animals placed in the same or a similar zinc cage, in the dark, and with a sufficient air supply. One of the main causes of variation in the total heat-liberation per 24 hours is the giving or the withholding of food. The total energy-exchanges are considerably higher and more irregular in animals which have been recently fed, than in animals which have fasted for (say) 24 hours. This fact will be considered in more detail below (Table II), but it must be remembered in a treatment of the experiments first to be described. The heat-production in all the

[1] This *Journal*, XLIV. pp. 427, 428. 1912.

experiments in Table I has been estimated in animals which have already been kept without food for 24 hours, and their weights were always taken immediately before the experiment began. By such means constant conditions are much more readily obtainable. It has been generally held since Rubner's work[1] on the relation of total metabolism to size, that the heat production of a warm-blooded animal is directly proportional to its body surface, the number of calories produced per sq. cm. per day being about 100. This "law" is reasonable, and obviously in many ways accords with the facts: doubt has however been thrown on its universal and exact validity. For example, Hopkins states[2] that his experience in dieting young rats leads him to the conclusion that the demand for maintenance is more nearly determined by the live-weight than by the body-area. The body-surface has been assumed to be proportional to the $\frac{2}{3}$ power of the body-weight[3], so that if H is the total heat set free by an animal of weight W, we should expect on Rubner's and Meeh's hypotheses that the ratio $H/W^{\frac{2}{3}}$ would be constant. In order to test to what degree Meeh's formula for the relation between surface and weight is correct in the case of the particular rats used we have made the following experiments. Five rats of weights 50·5, 62, 76, 82 and 129 grms. respectively were killed, and the areas of their skins measured and found to be 131, 162·5, 171·5, 194, and 251 sq. cms. respectively. The ratios of surface to weight, S/W, are in order, 2·59, 2·62, 2·26, 2·36, 1·94: while the ratios $S/W^{\frac{2}{3}}$ are 9·60, 10·37, 9·56, 10·27, 9·86 respectively. The latter ratios exhibit no considerable variations, so that one may reasonably assume the constancy of the ratio of the surface to the $\frac{2}{3}$ power of the weight. In this case the ratio of heat-production to body-area is well represented by $H/W^{\frac{2}{3}}$: or in absolute units, calories per sq. cm. (using the mean value 10·0 for the ratio $S/W^{\frac{2}{3}}$), by the quantity $\frac{1}{10}H/W^{\frac{2}{3}}$.

In all the following experiments of Table I are given (i) the weight of the animal after a 24 hours' fast, (ii) the temperature of the calorimeter during an experiment, (iii) the time of year, (iv) the heat-production H/W calculated per grm. of body-weight, (v) the heat-production calculated per (grms.)$^{\frac{2}{3}}$, *i.e.* $H/W^{\frac{2}{3}}$.

The animals all fasted 24 hours before the experiment began, and

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[1] See especially E. Voit, *Ztschr. f. Biol.* XLI. p. 120. 1901.

[2] This *Journal*, XLIV. p. 445. 1912.

[3] Meeh. *Ztschr. f. Biol.* XV. p. 425. 1879.

in nearly every case the latter was continued for 24 hours. During this second 24 hours' fast the heat-production is generally very regular, and does not fall noticeably. It represents the "maintenance" heat-production of the animal, necessary either to keep the animal warm, or as a consequence of the inevitable fasting metabolism of the living tissues. The fact that much larger quantities are obtained when the animals are fed shows how important it is to use certain standard conditions in investigating such problems as these.

In the following Table are given all the experiments (30) performed in this connection. There has been no selection of the experiments in order to obtain regular results. We have given the values both of the ratio of heat-production to body-weight, and of the ratio $H/W^{\frac{2}{3}}$. The experiments have been divided up into three groups, corresponding to the weights of the animals, viz. (1) the smallest animals under 90 grms., (2) the medium-sized animals between 90 and 130 grms., and (3) the large animals over 130 grms.

TABLE I. *Weight in grms.: heat-production in grm.-calories.*

Weight of animal	50·5	62	70	71	76	78	82	87·5
Temperature	14°	14°	14°	14°	15·5°	13°	15°	14°
Time of year	Aug.	Aug.	Dec.	Dec.	Aug.	Dec.	Aug.	Oct.
$H/W^{\frac{2}{3}}$	1234	1540	1279	1297	1500	1437	1497	1530
H/W	325	390	311	314	355	337	345	345

Weight of animal	96	103	103	117	118	118	122·5	124	127	129	129
Temperature	14·5°	14°	14°	14°	13·2°	14°	12·8°	13°	14·5°	14°	15°
Time of year	Oct.	Nov.	Aug.	Nov.	Sept.	Nov.	Sept.	Nov.	Oct.	Nov.	Aug.
$H/W^{\frac{2}{3}}$	1048	843	967	834	980	940	1194	960	1170	917	1035
H/W	229	180	206	171	200	192	241	193	233	182	205·5

Weight of animal	133	133	136	143	148	152	153·5	158	175	191	235
Temperature	14·5°	12·4°	15°	15°	12·8°	11°	14·5°	12°	13·5°	15°	12°
Time of year	Oct.	Sept.	Oct.	Oct.	Sept.	Oct.	Oct.	Nov.	Nov.	Oct.	Sept.
$H/W^{\frac{2}{3}}$	1126	1115	1158	1000	1055	1122	945	1100	1162	1160	1152
H/W	221	219	206	192	200	211	177	204	208	202	187

From this Table we see that the ratio $H/W^{\frac{2}{3}}$ is not very constant, neither is the ratio H/W very constant. We find that the ratio $H/W^{\frac{2}{3}}$ in group (i) has an average 1400, in group (ii) 990, in group (iii) 1100. There are sufficient experiments in each group to make the average fairly trustworthy, so that one may safely conclude that the ratio of heat-production to surface is not constant, but shows variations as great as 40 % of the whole. It is striking also that the ratio, as we increase the size of the animals, does not uniformly increase or decrease, but first decreases very considerably, and then increases again, though to a smaller value.

The ratio H/W on the other hand exhibits a certain degree of constancy: in group (i) it averages 340, in group (ii) it averages 203, and in group (iii) it averages 202·5. Thus in larger animals, those between 90 and 235 grms., *i.e.* from one-third to full grown, there is very exact constancy of H/W: the fasting heat-production is directly proportional to the body-weight. In the case of very small animals the ratio H/W is larger, partly we suppose because of the greater activity and restlessness of these young animals, and possibly partly also on account of their relatively greater surface. That it is not due only to relatively greater surface is obvious from the fact that $H/W^{\frac{2}{3}}$ for small animals, viz. 1400, is also considerably greater than it is for large animals, viz. than 1100. There must be some factor at work other than relatively greater surface, in order to bring the value of the fasting heat-production to such a high value for the small animal. This high heat-production must in fact be due to the animal being young, as well as to its being small. Indeed if we pursue the argument to its logical conclusion, and assume that the smallest ratio $H/W^{\frac{2}{3}}$ which can keep the animal warm is that shown by group (ii), viz. 990, then we must suppose that the starving metabolism of the young animal is quite independent (at 14° C.) of its heat loss, being on the average 40 % greater than would be sufficient merely to keep it warm.

It is striking in this connection that the values of the heat-production per sq. cm. of body-surface per day as given by E. Voit[1], and deduced from Rubner's and Meeh's hypotheses, agree very closely for various species of animals with the mean value 129 we have obtained[2] from Table I. For example, Voit found for the horse 95 cal., for the pig 108 cal., for man 104 cal., dog 104, rabbit 78, goose 97, fowl 94, and mouse 119.

We come next to the effects of food upon the total metabolism. In the first place we have estimated the total heat output of animals which have been allowed to eat as much food as they desired during the course of the whole 24 hours of the experiment. They do not eat possibly quite as much food as normally, since they are in absolute darkness in the calorimeter, and no doubt their appetite is lowered by the fact that they spend most of their time sleeping. Certainly,

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[1] *Op. cit.*

[2] The mean value of $H/W^{\frac{2}{3}}$ is 1287, from Table I. $S/W^{\frac{2}{3}}$ is 10·0, hence H/S is 128·7, or 129 approx.

however, they do eat as much as they desire, the darkness not directly hindering them from finding the food. The results of these experiments are shown in Table II. The temperature as in the experiments of Table I was regularly about 14° C.

TABLE II. *The effects of food in increasing the total metabolism of rats or mice living at about 14° C. in the dark.*

Exps. 1 to 6. Rats. In Exp. 1 the animal was fed on cheese: in Exps. 2 to 6 on damp biscuit.

Number of experiment	1	2	3	4	5	6
Weight of rat	55·5	57·5	80	86	89	198
H/W	400	424	402·5	424	357	248
$H/W^{\frac{2}{3}}$	1524	1636	1731	1890	1590	1447

These experiments should be compared with those in Table I, made upon rats of the same weights respectively. The ratios H/W would have approximately the following values in order, for fasting rats of the same weights: 360, 360, 340, 340, 340, 203. These latter values are on the average about 13 °/₀ less than those for the animals which were allowed food.

Exps. 7 to 13. On mice, several together in the calorimeter. In Exps. 7 and 8 fasting: in Exps. 9 to 13 fed on biscuit.

Number of experiment	7	8	9	10	11	12	13
Number of mice used	3	17	11	8	4	4	6
Average weight	26·5	6·8	12·5	10·7	19·5	20	20·5
H/W	939	920	1275	1496	1422	1755	1572

A comparison of Exps. 7 and 8 with Exps. 9 to 13 shows at once that the total metabolism of the fasting animals is much lower, at 14° C., than that of animals which are allowed food.

From Table II we see that animals which have been fed during the experiment, although they have not eaten very large quantities, show considerably more heat production than do fasting animals under identical conditions—as much on an average as 13 °/₀ more for rats, and more still for mice.

Let us turn next to experiments which show the gradual decline, and the fall to a constant value, of the heat-production of these animals after their last meal, and as fasting continues.

From these experiments we see that the fall which comes on during fasting in the rate of total heat-production lasts for about 24 hours, and that then the heat-production remains pretty constant. It is of course true that this fall appears rather larger here than Table II might lead one to expect. This is no doubt due to the fact that the animals become more quiet and contented in their new surroundings as time goes on. That the whole of the fall is not due to this latter fact is shown by the curves of total heat-production of animals which were placed in the flasks after they had already fasted for 24 hours. In the sixth eight hours after fasting began, *i.e.* in the third eight hours in the calorimeter, the heat-production was (in 16 experiments) only about 10 °/₀ less, on an average, than in the first eight hours in the calorimeter. This 10 °/₀ fall is much smaller than that occurring in Table III, so that accommodation to their surroundings in the calorimeter does not explain the total fall in metabolism during fasting. The results given in Table II show a 13 °/₀ fall in metabolism during fasting: no doubt, but for the fact that the animals became quieter in their new surroundings, Table III would show about the same figure.

TABLE III. *Effects of fasting on the total metabolism.*

Exp. 1. Three rats together, 95, 89, and 82 grms. respectively. In successive periods of seven hours the heat-production reckoned in calories per grm. per day was 208, 187, 182, 178, 175, 176: it will be observed that after about 24 hours the heat-production became constant at about 15 °/₀ less than the original value.

Exp. 2. Two large rats, 199 and 214 grms., together.

With food	245·5	cal. per grm. per day
First day's fast ...	191	,, ,, ,,
Second day's fast ...	163	,, ,, ,,

A fall in the total metabolism of over 33 °/₀.

Exp. 3. Three large mice at 16° C.: average weight 26·5 grms. before starving. In successive periods of eight hours after their last meal the heat-production reckoned in calories per grm. per day was 432, 368, 329, 287 respectively, a total fall in a day of 33 °/₀.

Exp. 4. Three young rats at 14·5° C.: average weight 54 grms. before starving. H.-P. in successive periods of eight hours, 249, 225, 194, 186, 198, 184. The mean of the last three periods, viz. 189, is 24 °/₀ less than the original value.

What then is the explanation of this fall? No doubt it can be laid to the credit of the same factor as Rubner[1] has described under the term "specific dynamic energy of foodstuffs." It should be noted however that when the animal's total metabolism is determined by its heat loss the "specific dynamic action" of foodstuffs has no influence on the total heat-production: Rubner's[2] calculation (if the hunger minimum be taken as 100), that on a diet of protein the total heat-production would be 140, on a diet of fat 114, and on a diet of cane sugar 106, is concerned only with an animal living at an external temperature of 33° C. In such an animal there is no need of any extra metabolism to

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[1] Rubner. *Energiègesetze*, p. 145, etc.

[2] *Op. cit.*

keep up the body temperature, the starving minimum for the life processes being more than sufficient for this latter purpose. The animals we used were fed upon biscuits, of composition we may assume to be about 13 % protein, 85 % carbohydrate, 2 % fat. Assuming Rubner's numbers for the specific dynamic actions of these several constituents we should expect an extra heat-production of about $[13 \times {\cdot}31 + 85 \times {\cdot}06 + 2 \times {\cdot}13]$ per cent., *i.e.* about 9 %. This is actually less than the value of 13 % observed. The interest of this is considerable. Were the animal really dependent on its heat-loss for the regulation of its total energy exchanges at 15° C., then we should expect that this 13 % produced by the "dynamic action of the foodstuffs" would be used in place of other heat to keep the animal warm. The fact that it is not so used, that at 15° C. the animal gives out 13 % more heat when fed, certainly confirms very strongly the conclusions following Table I, viz. that the heat-production of rats at 15° C. is not directly proportional to, or determined by their body surface, but is a necessary consequence of the chemical activities of their tissues.

We come then finally to the effects of keeping several rats together, in lowering their metabolism per grm. of body-weight per day. Hopkins[1] has described an effect he has often observed with rats, viz. that with a given food consumption they grow better when kept together in pairs than when kept separately. The explanation we have found of this fact is that animals when kept together give out very much less heat than when apart. If a young animal on a given diet gives out less heat, it will presumably have more food substance at its disposal for processes of growth. The explanation of this lowered value of the heat-production is not quite obvious, but the fact itself is quite clearly shown in Table IV. In calculating the heat-production per unit area, from the formula $H/W^{\frac{2}{3}}$, it is necessary to remember (*a*) that if several animals are present this gives the required quantity only if W is the average weight of one animal, and H the average heat-production of one animal, and (*b*) that the formula is even then justifiable only if all the animals used are of approximately the same size.

The average of H/W in all animals less than 90 grms. (group (i) above) is here 206 instead of 340[2]: for all animals over 150 grms. (group (iii)) H/W is 171 instead of 203. It is seen therefore that in young and growing animals the heat production of two or three animals living together is some 40 % less than that of the same animals living apart.

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[1] This *Journal*, XLIV. p. 427. 1912.

[2] See p. 97, above.

This enormous lowering of the heat-production means a very great increase in the possibility of growth upon a limited diet; in nutrition experiments therefore great care must be taken that animals either (*a*) always live in pairs, or (*b*) always live separately. It is noticeable that the effect of living in pairs is much less marked in large full-grown animals, for it leads to a fall of only about 16 %, instead of 40 %, in the total heat-production.

TABLE IV. *Heat-production of animals living together.*

Rats only were used. Each experiment lasted for 24 hours. The animals all fasted 24 hours before the experiment began, as well as during the experiment itself. They are therefore exactly comparable with the rats used in Table I.

Number of experiment	1	2	3	4	5	6	7	8	9	10
Number of rats used	4	3	3	2	2	2	3	3	2	2
Average weight W	52	54	68	80·5	81·5	85·5	89	150	190	192·5
H.-P. per grm., H/W	217	213	220	177	205	217	193	162	177	173
$H/W^{\frac{2}{3}}$	801	805	895	762	891	958	862	862	1020	1000

Now as regards the physiological explanation of this lowering of metabolism, a very plausible explanation was originally suggested. Whether it will be found satisfactory, in view of the experiments described already in this paper, we very much doubt. When animals live together, especially in the dark, they present a very much smaller surface for heat loss than they do when living apart: in the dark they are usually nestling up against one another, and consequently lose less heat. Two animals curled up close together may roughly be taken to have the same surface as one large animal twice the size. If we calculate the value of the heat-production per unit of surface on this assumption we find, instead of the values given in Table IV, viz. 810, 805, 895, 762, 891, 958, 862, 862, 1020, 1000, the values, 1283, 1160, 1290, 957, 1120, 1207, 1240, 1240, 1283, and 1260. It is at once obvious that the latter values for the ratio of heat-production to body-surface are much more nearly like those given in Table I than are the former. The latter average 1204: the former only 887: while the average for all the values of $H/W^{\frac{2}{3}}$ given in Table I is 1287. This fact suggested very strongly that in young animals the reason why there is less heat-production per grm. of body-weight when they are kept together than when they are kept apart, is that when together they present a very much smaller surface for heat-loss. The main objection to this rather pretty hypothesis is that the results of all the other experiments in the paper are against the belief that the heat-production of rats at 15° C. is determined by a loss of heat proportional to their body-surface. If this latter is indeed the case, the foregoing explanation cannot hold. It is indeed possible that the reason for the low heat-production of animals living in pairs is that they are less active, that they sleep better, and are more comfortable if they have a neighbour. It seems to us that, all the facts considered, the latter hypothesis is the more plausible; and that the approximate equality of the two values 1204 and 1287 given above is no more than a coincidence. It may be however that at some temperatures the decrease of exposed area due to keeping animals together may have some considerable influence on the total energy-exchanges of the animals.

But be the explanation what it may, the fact itself, the lowered heat-production of animals living in pairs, must be of importance in work upon animal-nutrition.

In conclusion we may add that we hope in future to use this calorimeter for investigation of the total energy-exchanges of small animals under various conditions, external or dietetic. At present and for some time past, one of us (A. M. H.) has been using it for an investigation of the total heat-production of animals fed upon a synthetic diet[1] free from various constituents necessary for growth.

CONCLUSIONS.

1. A description is given of an automatic self-registering respiration-calorimeter for small warm-blooded animals, by the use of which it is possible to estimate within about 2 % the total heat liberated by the animals. A complete record of the heat-production at every moment can be obtained for any period desired, up to several days.
2. In experiments at about 15° C. upon fasting individual rats, kept without food for 24 hours before being placed in the calorimeter, it was found that the ratio of heat-production to body-weight (heat-production per grm.) is quite constant for all those rats which are more than one-third the size of a full-grown animal: for smaller animals than this the heat-production per grm. rises rapidly as we take smaller and smaller animals, and may be 70 % greater than for grown animals.
3. The body-surface being S sq. cm. and the body weight being W grms., the ratio $S/W^{\frac{2}{3}}$ in rats is constant, and equal to the number 10: hence $S = 10 \times W^{\frac{2}{3}}$ for rats.

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[1] See Hopkins, *op. cit.*

4. The ratio of heat-production to body-surface in fasting animals at 15°C. is not constant, being 140 cals. per sq. cm. for small animals, 99 cals. per sq. cm. for medium-sized animals, and 110 cal. per sq. cm. for large animals. Any assumption that the heat-production of a rat at 15°C. is determined by the heat-loss is therefore unjustifiable.

5. It is suggested that the very high value of the heat-production per grm. at 15° C. of rats which are less than one-third grown, is due, not to the fact that they are small and have a relatively larger surface, but to the fact that they are young: and that even though fasting the chemical activities of their tissues are higher than in older animals, and are more than sufficient to keep the body warm.

6. Rats fed on biscuit at 15°C. give out about 13 °/₀ more heat than similar animals fasting: as fasting proceeds after the last meal the heat-production per grm. falls continously for 24 hours and reaches then a constant value. If this 13 °/₀ difference is due to the "specific dynamic action" of the foodstuffs (Rubner), its occurrence suggests that the animals are, even when fasting, giving out more heat than is necessary to keep them warm: in fact it supports the contentions (4) and (5) above that the total metabolism of a rat at 15°C. is not regulated by its heat-loss per unit of body-surface.

7. For animals kept together in twos or threes in the calorimeter at 15°C. the heat-production per grm. is very much less (as much as 40 °/₀ in young animals) than it would be if the animals were kept separately. The suggestion that this lessening of the heat-production is due to the smaller area exposed to heat-loss, in the case of two animals resting close to one another, would account with some degree of accuracy for the degree of lessening observed. This idea is, however, not in keeping with conclusions (4) (5) and (6) above. It is suggested therefore that it must be due to the lessened activity of animals living in pairs, to the fact that they sleep and rest more quietly when they have a companion. Be the explanation however what it may, the fact itself must be of considerable importance in the breeding and nutrition of young animals: for with a given diet, if the heat-production is less when the animals live together, then under such conditions they will grow faster. That animals on a given diet do grow faster when living together has already been shown by Hopkins.

The thread recorder employed was obtained by Dr F. G. Hopkins, by means of a grant from the Royal Society: the expenses of the research were largely defrayed by another grant from the same source, to one of us (A. V. H.).