Botany. — *An automatic micro compensation-calorimeter.* By L. ALGERA. (Communicated by Prof. J. C. SCHOUTE.)

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Although it has long been known that plants, like animals, give up, during the vital processes, part of their energy in the form of heat to their environment, very few accurate measurements of this have as yet been made. The reason for this is the lack of a good method, this being due principally to the fact that the amount of heat produced by plants is as a rule much smaller than that produced by animals, so that an exact determination is attended with much greater difficulty. Another condition requisite for a good method and which is difficult to fulfil is the possibility of a long duration of the test. Until this is attained it will be impossible to form a complete idea of the course of this yielding-up of heat. In most of the existing methods the duration of the test is rather limited, so that only a fragmentary impression of the production of heat is obtained.

In order to satisfy both of the requisite conditions, a calorimeter was constructed which accurately measures even a very slight production of heat, and which at the same time performs the measurement entirely automatically [1]). This calorimeter was found to be very suitable for following the course of the production of heat in the case of Aspergillus niger, which plant was chosen for the experiment, from the germination of the spores to any desired stage.

The principle on which the method is based is that of compensation of the heat developed by an equal amount of generated cold. For this purpose there is in the culture vessel, in addition to the heat producing object, a source of cold. This source of cold is a thin tube filled with distilled water, through which dry air can be pumped. During the evaporation which then takes place the requisite cold is generated. As soon as the temperature of the culture vessel rises, in consequence of the giving-up of heat by the organism, the pump is automatically set in action and pumps the dry air through the water. As soon as the temperature of the culture vessel has fallen, as a result of this, to just below the original temperature, the pump is automatically stopped. Owing to the uninterrupted production of heat by the organism, the temperature again rises, the pump is again started, and so on. If care is taken to secure accurate compensation, then the amount of heat produced is equal to the cold generated. This latter

[1]) I am very grateful to Prof. Dr. F. ZERNIKE and Drs. T. DIJKSTERHUIS for their assistance in matters of physics, and I am also greatly indebted to JAC. VEENHOFF for the manner in which he prepared various parts of the apparatus.

corresponds to a certain amount of evaporated water. The water evaporated is determined, at a given temperature, by the volume of air pumped through, and this in turn by the number of strokes of the pump. If, therefore, the number of calories to which one stroke of the pump corresponds is known, it is a very simple matter to calculate the heat generated from the number of strokes required for compensation.

Now that the working has been explained in a few words, a more detailed description, based on a diagrammatic figure, may be given. The following points will be discussed in the order indicated :

A. The culture vessel.
B. The device for automatic compensation of the heat developed.
C. The determination and registration of the amount of such heat.
D. The accuracy of measurement.
E. Advantages and possibilities of the calorimeter.

A. The Culture Vessel.

This is a cylindrical, thin-walled copper vessel (*C*) of about 300 cubic centimetres content, closed by means of a lid. As already stated, it contains both the source of cold and that of heat. The source of cold (the evaporator) is a thin brass tube *E*, consisting of an upper, wide cylinder connected with a lower, much narrower one, and which at the beginning of the test is almost entirely filled with distilled water. The tubes *TI* and TO serve to convey the dry air to and from the evaporator respectively. The air, in passing through the water, becomes saturated with water vapour, thus generating the cold required for compensation. As a result of the widening of the evaporator, the level of the liquid falls only to a very slight extent through the evaporation of the water.

Both the above-mentioned tubes also serve as supports for the evaporator in the culture vessel. This in turn supports the boxes in which the objects grow. These boxes are cylindrical in shape, and have a hole pierced through the centre. This makes it possible to push them over the narrow part of the evaporator; the walls of the perforation close so firmly round the evaporator that the boxes cannot slip downward. This method of setting up was chosen with a view to obtaining as good an exchange of heat between the sources of heat and cold as possible. The differences of temperature are very quickly exchanged by way of the metal walls of the boxes and the evaporator, so that the inertia of the apparatus is restricted to a minimum. In order to increase this exchange there are also a couple of brass strips (not shown in the fig.) running from the wide part of the evaporator down the outside walls of the boxes. The breadth of each of these strips is one fourth of the external circumference of the boxes, so that the objects can absorb enough oxygen from the surrounding air and can give up carbonic acid. In order to determine not only the heat but also the gas metabolism, the air in the culture vessel forms part of a closed circuit, from which the carbonic acid formed is removed by

means of baryta water, the oxygen absorbed being replaced by fresh oxygen. This point is, however, not dealt with in this communication, so that the circuit is not included in the figure.

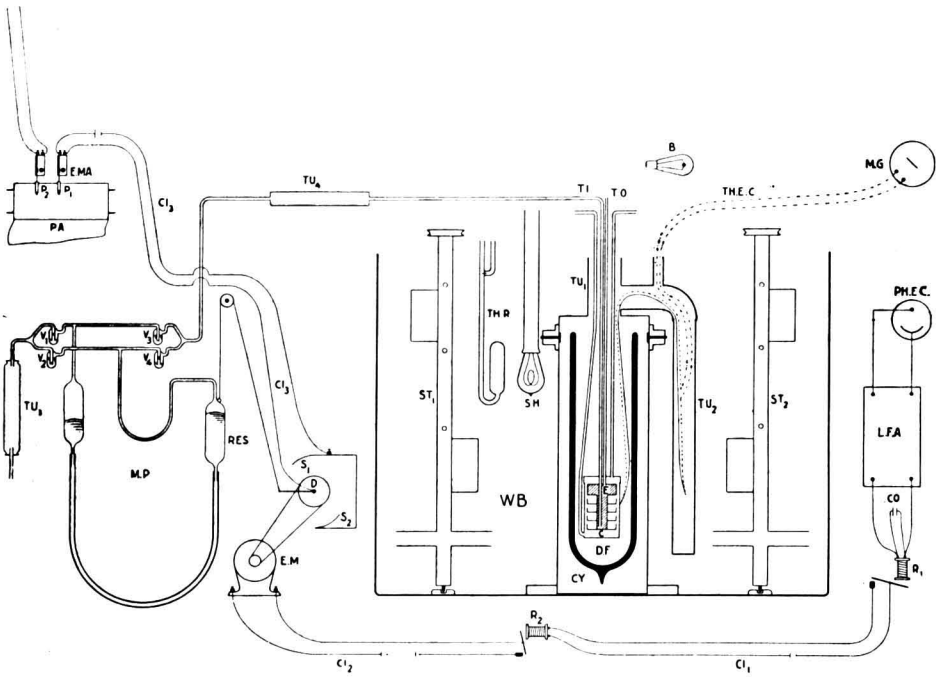

DIAGRAM OF THE CALORIMETER.

B = bulb; C = culture vessel; CI_1, CI_2, CI_3 = electric circuits; CO = condenser; CY = cylinder for Dewar flask; D = disc; $D.F$ = Dewar flask; E = evaporator; $E.M$ = electromotor; $E.M.A$ = electromagnet; $L.F.A$ = low frequency amplifier; $M.G$ = mirror galvanometer; $M.P$ = mercury pump; P_1, P_2 = pens; PA = paper; $PH.E.C$ = photo-electric cell; R_1, R_2 = relays; RES = reservoir; S_1, S_2 = springs; $S.H$ = source of heat; ST_1, ST_2 = stirrer; $TH.E.C$ = thermo-electric circuit; $TH.R$ = thermoregulator; TI = inlet tube; TO = outlet tube; TU_1 = tube; TU_2 = tube with paraffin oil; TU_3, TU_4 = tubes with P_2O_5; V_1, V_2, V_3, V_4 = valves; $W.B$ = water bath.

In order to reduce the exchange of heat with the environment to the smallest possible amount, the culture vessel is placed at the bottom of a Dewar flask 40 cm deep ($D.F.$). Although an excellent heat insulation is obtained in this way, prolonged variations in the temperature of the surrounding air made themselves felt in the Dewar flask. To prevent this it was built into a wider and higher brass cylinder CY and thus placed in the water of the bath $W.B$. The cylinder is closed by means of a lid that can be screwed off if the culture vessel has to be taken out of the Dewar flask. The lid is furnished with tube TU_1, which serves to admit the inlet and outlet tubes of the culture vessel. The tube is, however, as narrow as possible. No variations in the room temperature were therefore to be observed. The cylinder and the Dewar flask are thus practically entirely

surrounded by water, and the temperature inside is dependent solely on that of the water. The use of a feeble source of heat (carbon filament lamp *S.H*) and of a sensitive thermo-regulator (*THR*), which were fitted up in proximity to each other, in addition to vigorous stirring and a large volume of water, made it possible to limit the fluctuations in the temperature of the water to 0°.001 C. at the very most. Once an equilibrium of temperature has been reached, the culture vessel undergoes no further changes of temperature, since the rapid fluctuations of 0°.001, thanks to the insulation and heat capacity of the envelope, are unable to penetrate into the Dewar flask. This fact is also of importance in securing a correct heat compensation, as will shortly be seen.

B. *The device for automatic compensation of the heat generated.*

It should now be discussed how an accurate heat compensation can be obtained. The evaporation of the water in the evaporator must be so regulated that neither too much nor too little heat is taken away. The thermo-electric circuit *TH.E.C* acts as the first link in the chain which renders this possible. This consists of twice ten thermocouples connected in series, one group of which, in two series of five, is fixed to the side of the culture vessel by a couple of insulated brass strips. The second group is inside the metal tube TU_2, which is filled with paraffin oil. This is soldered on to tube TU_1 and is inserted into the same water-bath as the Dewar flask. In a state of equilibrium of temperature both groups of thermocouples have the same temperature, and there is no current in the circuit. Owing to the excellent insulation it takes some considerable time before the inside wall of the Dewar flask has reached the temperature of the water-bath. By taking the necessary precautions one can prevent the temperature of the flask from differing from that of the bath at the beginning of the test. Constantan and manganin were chosen as metals for the thermo-electric circuit. For clearness sake only one thermocouple of each group has been drawn in the fig. The continuous line represents a manganin wire, the dotted line one of constantan, and the dot-dash lines the connecting wires with the mirror galvanometer *M.G* which forms part of the circuit. This mirror galvanometer (Kipp type *V*) serves to throw a little light from the bulb *B* into the photo-electric cell *PH.E.C* (Philips N°. 3510). The photo-electric current thus obtained is amplified by the low frequency amplifier *L.F.A*, the terminal valve of which acts as a detector. The rapid variations of current requisite for the amplification of the current by the transformer are obtained by placing a toothed revolving disc (not shown in the fig.) at a suitable point in the beam of light. The light is now by turns intercepted and transmitted, so that a photo-electric current is produced, which constantly varies in strength. A frequency of approx. 200 per second was found to give the greatest amplification. In consequence of the action of the detector, current impulses of the frequency just mentioned are caused in the anode circuit of the terminal

valve, and the relay R_1 which is included in this circuit will therefore constantly receive current impulses. To obviate vibration the impulses are levelled down by the condenser CO connected in parallel with the relay. As soon as the direct current obtained in this way amounts to approximately 4 mA., the relay is attracted. If the strength of the current decreases to approx. 3 mA., it springs back again. The mirror galvanometer is set up in such a way that, in a state of equilibrium of temperature, so much light falls into the photo-cell that a current of 3½ mA. runs through the relay. If the temperature of the thermocouples rises, owing to the generation of heat by the objects, a thermo-electric current is produced which causes the mirror to turn a trifle to the left. Now more light falls into the photo-electric cell, and the anode current increases. When this has reached the value of 4 mA. the relay is attracted and the current circuit CI_1 is closed. In consequence of this, relay R_2 is attracted and circuit Cl_2 is closed. The electromotor $E.M$ included in this circuit is started, and drives the mercury pump $M.P.$ When disc D revolves the reservoir RES of the pump is moved up and down. The four valves V_1, V_2, V_3, V_4, cause air to be drawn forward from the left and pressed on to the right. Before the air reaches the pump it first passes through the tube TU_3 filled with P_2O_5. To make assurance doubly sure, the air is passed once more over P_2O_5 (tube TU_4), before it enters the evaporator. This perfectly dry air then passes through the water in the evaporator. The evaporation then taking place cools off the water and the wall of the evaporator, and these now withdraw heat from the objects and the culture solution. These fall in temperature, with the result that the thermocouples also become colder. The thermo-electric current becomes feebler; the mirror of the galvanometer turns back again, with the result that the anode current of the terminal valve decreases. As soon as this has reached a value of 3 mA., the relay springs back again and so causes the motor and pump to stop. The generation of cold is ended. In consequence of the uninterrupted generation of heat by the organism the temperature again rises; the cooling process is again started, and so on.

The fluctuations of temperature thus brought about are very small, and do not exceed the amount of 0°.013 C. In order to raise the anode current from 3½ to 4 mA., i.e., in order to set the compensation in action, a temperature of 0°.0008 C. is sufficient. Owing to slight inertia the anode current rises to at most 6 mA. It likewise falls during cooling to about 0. This variation of current corresponds to a temperature difference of 0.°013 C. It is now evident why the culture vessel was protected with such care from variations of temperature coming from outside. Not only would troublesome errors be caused, with such an accurate compensation, by the exchange of heat attendant on even slight changes of temperature, but such changes would also alter the temperature of the thermocouples and at the same time the anode current, thus causing the heat compensation to function incorrectly.

In spite of the good insulation an exchange of heat, even if only a very slight one, is possible. This is in practice reduced to nil by the fact that the mirror galvanometer is set up in such a way that the fluctuations of temperature occur symmetrically about the temperature equilibrium. The positive temperature deflections neutralize the effect of the negative ones. Owing to the good heat insulation even a very slight generation of heat is able to increase the temperature by 0°.0008 C., an amount that, as we have seen, is sufficient to bring about the compensation. *Such an increase is caused by a source of heat with a capacity of about 0.01 gram-calories per hour.* All this shows the great sensitiveness of the apparatus.

Seeing that with a heat capacity of the culture vessel of approx. 60 grams the deviation of temperature is no more than 0°.07 C., the amount of heat generated may at all times be considered equal to the cold generated. This fact was used to determine the value first mentioned, as will be seen from the following.

C. *The determination and registration of the heat generated.*

The amount of cold generated is proportional to the amount of water evaporated; the evaporation is determined by the volume of the air passed through. The air invariably contains the same quantity of vapour per volume unit, since the temperature of the water is practically constant, and in any way fluctuates about a constant average, and further since the level of the liquid in the evaporator falls very little. Finally, the number of pump strokes required is a measure of the air volume. The cold produced (= heat developed) can now be calculated if the following data are known:

a. The number of pump strokes required for compensation.
b. The number of calories that are compensated by one pump stroke (caloric value of one pump stroke).

a. The first value is determined in two ways. In the first place by means of a cyclometer attached to the shaft of disc *D*. From this the number of pump strokes can always be read off. The second method consists of a registering device, by means of which not only the total number, but also the distribution of the strokes over the time is noted. The registration takes place in the following manner.

Every time the arm of disc D touches one of the two brass springs S_1, S_2, while pumping is in progress, the circuit CI_3 is closed and the electro-magnet *E.MA* attracts the pen P_1. When the circuit is open this draws a straight line on a strip of unrolling paper *PA*. If the circuit is closed for a moment, a lateral deflection of the line takes place. The figure shows that contact is made twice with each pump stroke, so that the number of deflections must be divided by two to obtain the number of pump strokes. By allowing contact to be made twice one obtains a more accurate registration than would be the case if contact were made once. A second similar pen P_2 is connected with a clock and gives a time-

signal at every quarter. By comparing the lines traced by the two pens, one can follow the course of the generation of heat in the time, whether during or after the test. The cyclometer serves principally to keep one posted as to the total number of strokes even if registration ceases for some reason.

b. The caloric value of one pump stroke is determined by gauging. In the boxes for the fungus a thin manganin wire can be fixed, connected by means of a couple of thick wires with a 2-Volt accumulator. In order to obtain a good generation of heat, a resistance box outside the culture vessel was further included in the circuit. (This circuit is not shown in the fig.) If we say that :

r_1 = resistance in the box,

r_2 = resistance of the leads,

r_3 = resistance of the manganin wire and

v = terminal potential difference of the accumlator,

then $0.239 \times \frac{v^2}{(r_1+r_2+r_3)^2} \times r_3 \times 3600$ gram-calories are generated per hour in the culture vessel.

r_2, r_3 and v were determined very accurately. If the amount of heat generated is thus known and the number of pump strokes required for compensation has been read off, then the two numbers divided into each other gives the number of calories that are compensated by one pump stroke. From a large number of tests an amount of 0.924 gram-calories was found for this.

D. The accuracy of the measurements.

The gauging tests served not only to determine the capacity of one pump stroke, but also gave a notion of the accuracy of the measurements of the heat. As it is sufficient to know the limits of error with the amounts of heat given off by the object tested, this production of heat had to be known before the tests began. For this purpose the boxes in the culture vessel were filled with culture solution and sown with a number of spores of Aspergillus niger, after the caloric value of one pump stroke had been approximately determined from a few tests. At first the production of heat was nil, but it rose in the course of the two following days to approx. 40 gram-calories per hour, and remained constant for six days, after which the test was broken off.

On the strength of these data the gauging tests were now carried out with heat productions of 40, 20, 10, and 5 calories per hour. In view of the fact that one of the objects of the later tests will be to compare the production of heat with the gas metabolism, and this latter will be determined every two hours, it was evidently advisable to continue the gauging tests for periods of about two hours.

The following table I gives the results of the gauging tests with a generation of heat of about 40 cal. per hour.

TABLE I.

Date	Duration of test	Number of calories	Number of pump strokes	Caloric value of 1 pump stroke	Average per day
13—5—31	125′27″	80.69	87	0.927	
	117′6″	75.25	81	0.930	
	114′22″	73.56	79	0.931	0.930
	130′22″	83.85	90	0.932	
	108′45″	69.94	75	0.933	
15—5—31	118′29″	76.05	83	0.916	
	103′32″	66.45	72	0.922	
	93′6″	59.75	64	0.934	0.925
	76′20″	48.99	53	0.924	
	121′28″	77.89	84	0.927	
	120′	77.02	83	0.928	
16—5—31	127′3″	81.44	88	0.925	
	102′40″	65.81	71	0.927	
	82′22″	52.80	57	0.926	0.927
	91′7″	58.41	63	0.927	
	144′34″	92.67	100	0.927	
	139′15″	89.26	96	0.930	
18—5—31	121′28″	80.47	88	0.914	
	105′18″	69.76	76	0.918	
	103′12″	68.37	74	0.924	0.919
	89′50″	59.51	65	0.916	
	118′2″	78.20	85	0.920	
	124′3″	82.18	89	0.923	
19—5—31	104′25″	69.16	75	0.922	
	104′35″	69.27	75	0.924	
	102′45″	68.05	74	0.920	
	107′17″	71.06	77	0.923	0.922
	134′48″	89.28	97	0.920	
	107′34″	71.24	77	0.925	
20—5—31	105′14″	69.28	75	0.924	
	91′23″	60.16	65	0.926	
	103′27″	68.11	74	0.920	
	107′37″	70.83	77	0.920	0.921
	92′	60.57	66	0.918	
	116′	76.37	83	0.920	
	167′46″	110.45	120	0.920	

$M = 0.924$; $\sigma = \pm 0.0049$ or $0.53\,\%$; $m = \pm 0.0008$ or $0.09\,\%$.

The tests show that the error made with the two-hour determination of the production of heat is not more than 1.59 % of the heat produced during that time. For only 3 $^0/_{00}$ of the measurements is theoretically outside $M \pm 3\sigma$. As a rule the error is smaller. If we sum up the observations of each day and then calculate the day averages, we see that these averages, as was to be expected, differ much less from one another than the two-hour values. This proves that the total heat generated e.g. during a week is measured with still greater accuracy than that during the two-hour periods.

The general average of the caloric value of one pump stroke is found, with a production of heat of about 40 cal. per hour, to amount to 0.924 cal.

The tests with a production of heat of approx. 20 cal. per hour yielded the following result (Table II).

TABLE II.

Date	Duration of test	Number of calories	Number of pump strokes	Caloric value of 1 pump stroke	Average per day
21—5—31	120′55″	43.07	47	0.916	
	102′45″	36.60	40	0.915	
	108′12″	38.54	42	0.918	
	103′10″	36.75	40	0.919	0.917
	113′19″	40.35	44	0.917	
	128′45″	45.86	50	0.917	
22—5—31	124′6″	44.04	48	0.918	
	115′23″	40.98	45	0.911	
	95′14″	33.74	37	0.912	
	98′40″	35.05	38	0.922	0.913
	135′48″	47.94	53	0.910	
	162′12″	57.61	63	0.914	

$M = 0.915$; $\sigma = \pm 0.0034$ or 0.37 % ; $m = \pm 0.001$ or 0.10 %.

We see that with 20 cal. per hour the error is at the most 1.11 %. Further that the cal. value of one pump stroke is 0.915 cal.

The results of the gauging tests with a production of heat of about 10 cal. per hour are shown in Table III.

The tests show that the limits of error are 2,28 %; the caloric value is 0.933.

TABLE III.

Date	Duration of test	Number of calories	Number of pump strokes	Caloric value of 1 pump stroke	Average per day
8—6—31	98′18″	16.47	18	0.915	
	99′45″	16.71	18	0.928	
	116′48″	19.56	21	0.932	
	84′39″	14.18	15	0.945	0.931
	122′23″	20.50	22	0.932	
	117′5″	19.61	21	0.934	
9—6—31	112′7″	18.67	20	0.933	
	122′50″	20.45	22	0.930	
	90′	14.99	16	0.937	0.935
	113′1″	18.82	20	0.941	
	134′18″	22.36	24	0.932	
	123′51″	20.62	22	0.937	

$M = 0.933$; $\sigma = \pm 0.0071$ or $0.76\,\%$; $m = \pm 0.002$ or $0.22\,\%$.

With about 5 cal. (Table IV) the limits of error are 2.73 %. The caloric value is 0.916.

TABLE IV.

Date	Duration of test	Number of calories	Number of pump strokes	Caloric value of 1 pump stroke	Average per day
10—6—31	126′29″	10.84	12	0.903	
	118′3″	10.11	11	0.919	
	129′24″	11.09	12	0.924	0.916
	85′2″	7.28	8	0.910	
	107′12″	9.16	10	0.916	
	129′11″	11.07	12	0.922	
11—6—31	117′53″	10.07	11	0.916	
	117′1″	10 01	11	0.910	
	87′49″	7.51	8	0.938	0.916
	85′2″	7.27	8	0.909	
	161′9″	13.78	15	0.918	
	127′22″	10.89	12	0.908	

$M = 0.916$; $\sigma = \pm 0.0083$ or $0.91\,\%$; $m = \pm 0.0024$ or $0.26\,\%$.

It is difficult to say with certainty what the cause is of the differences in the caloric values with 40, 20, 10 and 5 cal. per hour. However this may be, it is obvious that any causes acting as sources of error will affect the value of a pump stroke according as the source of heat is feebler. It is therefore safest to assume as the value of one pump stroke the amount that was found with 40 cal. per hour, viz., 0.924 cal.

E. Advantages and possibilities of the calorimeter.

In comparison with the already existing methods, the calorimeter here described has various advantages. In this communication nothing further will be said as to these methods. One of them, however, should be made an exception of, since it is in principle similar to the one under discussion to the extent that the heat generated is taken away by means of a source of cold. The method in question is that of WAGNER.[1]) The apparatus consists of a couple of Dewar flasks, one of which is used to accommodate the animal to be experimented on, and moreover contains a spiral cooler. In both flasks there is a closed reservoir filled with air, which reservoirs are connected by a tube containing a drop of petroleum. If the temperature in the first flask rises owing to the production of heat by the animal to be tested, the drop shifts. Cold water is now passed out of a third Dewar flask through the spiral condenser until the drop has again taken up its original position, so that the initial temperature is re-established. By constantly repeating this, one can obtain a good heat compensation. The variation of temperature of the water flowing into and out of the spiral and the quantity of the water flowing through are measured. The heat produced is then equal to the product of the values measured.

The gauging tests carried out by WAGNER show that with a production of heat of approx. 400 — approx. 1000 cal. the greatest deviation of the values found from those calculated is 5.3 %. Although the production of heat was greater than in the tests described above, the limits of error are wider. The accuracy is therefore not so great. Furthermore, WAGNER's method is not automatic, and therefore ill-suited to a long duration of the test.

In addition to the advantages of great accuracy and automatic measurement of heat, those advantages are also obvious which result from the fact that the temperature is always kept constant (apart from the very slight fluctuations of temperature). There are three of these.

a. It is unnecessary to know the heat capacity of the organism. With all methods in which an increase of temperature occurs, this value has to be determined. This determination is as a rule very inaccurate, chiefly because the heat capacity during the test is generally subject to alterations.

[1]) R. WAGNER. Handbuch d. biol. Arb. methoden von E. ABDERHALDEN, Abt. IV, Teil 10.

A knowledge of the heat capacity of the culture vessel is likewise superfluous.

b. The correction of water evaporation can be eliminated. For this purpose it is only necessary to lead the air, before it enters the culture vessel, through a flask with culture solution, standing in the water-bath. The vapour tension in the circulating air is then equal to that prevailing above the culture solution in the culture vessel. Alterations of concentration of the latter during the growth of the organism have but little influence on the vapour tension.

c. The organisms are invariably grown under the same conditions of temperature, which may be desirable in view of the extent to which the vital processes depend on the temperature.

Another advantage is that, owing to the great rapidity with which the thermocouples react to variations of temperature in the culture vessel, an inconstant heat production is measured with the same accuracy as a constant or only very slowly varying production of heat. This is not the case with most of the existing methods, owing to the more or less sluggish adjustment of the temperature.

Finally the simplicity of the calculation may be mentioned; this consists solely in a multiplication of the caloric value of a pump stroke by the number of strokes required.

And with regard to the possibilities of the calorimeter, it may be noted that it can not only serve for the determination of the production of heat by Aspergillus niger, for which it was constructed, but can also, after the necessary modifications in the culture vessel, for other plants and animals. In case one evaporator is not sufficient for compensation, several of such may be placed in the culture vessel. Moreover the capacity of a pump stroke can be raised by substituting alcohol for water as evaporating liquid. In consequence of the much greater vapour tension, about 2½ times as much cold is generated with this per volume unit of air passed through.

In this short communication only the most important points could of course be touched upon. I will go into the matter in more detail elsewhere.

This investigation was carried out in the Laboratory for Plant Physiology at the State University at Groningen. I wish to express my hearty thanks to Prof. Dr. W. H. ARISZ for his help and interest, and for the great willingness with which he placed the necessary instruments at my disposal.