

A NOTE ON THE APPLICATION OF BUCHANAN'S
FORMULA TO HEAT PRODUCTION IN
BACTERIAL CULTURES

NORMAN C. WETZEL

*From the Department of Pediatrics, Western Reserve University Medical School,
and the Babies and Childrens Hospital, Cleveland, Ohio*

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This note is presented in order to discuss the results which Bayne-Jones and Rhees (1929) report in applying Buchanan's formula to their data on the production of heat in bacterial cultures. Unfortunately, the value of these observations is impaired by the fact that the mathematical treatment of the data is not entirely free from error. Apart from this, however, it appears desirable to reconsider the analytical aspects of the problem especially because the rate of heat production of a single bacterium cannot, contrary to the authors' claim, be represented in their experiments by the Buchanan formula.

They have based their claim entirely upon a striking numerical agreement between values calculated from the formula and those determined somewhat more directly from the individual measurements. The proof that this agreement is unreal will prepare the way for the demonstration that the liberation of heat had not taken place in accordance with the hypothesis from which Buchanan's formula has been derived.

We may begin our investigation of the matter by quoting from the original paper:

The heat produced by each bacterium was obtained directly by dividing the total gram calories by the number of bacteria present in the 100 cc. of culture medium.

This clearly defines one of the units with which we shall be required to deal, namely, the quantity of heat liberated *per bacterium*. Thus calculated, it has been considered an "ob-

served" value, since it was determined from the heat, and from the number of organisms experimentally measured and counted, respectively, at the end of each time interval,—in the present case, one hour. The highest value has consistently been found at the end of the second hour, and appears to be taken without further qualification as the true maximum of the function. Interpolation, however, on both growth and heat curves shows that the maximum is reached considerably earlier than the second hour. This needs to be mentioned only because the authors themselves have considered the discovery of points of inflection a rather significant result of their analysis. What concerns us more at the moment, however, is the fact that one of the units entering into the discussion has now been defined as *heat per bacterium*.

The sentence following that already quoted reads:

This value, of course, was actually too small to be measured but some interesting relationships were discovered through the graphic use of it.

The preceding statement thus refers unmistakably to the quantity heretofore defined as *heat per bacterium*.

We are, thereupon, informed that,

The same fact could be reached by a calculation.

The foregoing quotation obviously, can be interpreted only to mean that the dimensions of the quantity calculated from the formula which the authors are about to derive will be identical with the dimensions of the unit previously defined, that is to say, with *heat per bacterium*. This, however, is not the case, as we shall see by repeating the significant steps of the argument somewhat more briefly than in the original. It is assumed:

- (a) That "the organisms are multiplying in geometric progression at a definite rate";
- (b) That "each organism is excreting substance or (producing heat) at a definite uniform rate";
- (c) That heat is measured at the end of each unit of time;
- (d) That bacteria are counted at the beginning and at the end of each unit of time.—

Now, if, B = number of bacteria at onset,
 b = number of bacteria at t ,
 g = average generation time,
 S = total heat produced in t ,
 and, m = amount of substance or heat produced per cell per
 unit of time,
 then, by condition (a) above,

$$b = B2^{\frac{t}{g}} \quad (1)$$

if the "ratio in the geometrical progression" is 2.

Next follows the statement that "the amount of substance (or heat) produced during any instant dt would be $m b dt$," from which, (if this is true),

$$S = \int_0^t m b dt = mB \int_0^t 2^{\frac{t}{g}} dt \quad (2)$$

In view of some of the results which the authors have announced, it is important in the first place to note that m has been removed from under the integral along with the constant B , this being justified, of course, on the basis that "each organism is excreting substance, (or producing heat) at a definite uniform rate." The foregoing procedure and definition thus afford absolute evidence that m is to be understood constant. Here is a conclusion of the utmost importance to which we shall need to refer at a later stage of our discussion. But there is another matter that should also be carefully noted at this time. While it is true that the above equation gives us a perfectly plausible relation between total heat, the number of organisms expressed as a function of the time, and time itself, we must bear clearly in mind that this relation is by no means a necessary one. Put into words equation (2) tells us that S , the total heat liberated in time t , has been supposed directly proportional to the number of organisms present in the culture medium. As we have already pointed out, however, the results of the present study do not support that hypothesis; but, rather than complete our examination of this matter here we may proceed instead to evaluate the

right hand side of (2), which becomes upon integration, substitution from equation (1), and rearrangement of terms,

$$m^* = \frac{S 2.303 \log_{10} \frac{b}{B}}{t(b - B)} \quad (3)$$

This equation is known as Buchanan's formula, and serves as the basis for the "theoretical" determinations of the unit rate of heat production. Accordingly, when the authors now propose to demonstrate the applicability of this formula to their data by a comparison of the results so obtained with those determined by experiment, we should expect that the values of m would be compared with values representing the unit rate of heat production estimated from direct observation of heat output. Unfortunately, however, this has not been done. What has happened is that the values calculated from this formula have instead been compared with those values which we have already shown to be expressed in terms of *quantity of heat per bacterium* and not in terms of *rate of heat production per bacterium*. Here we refer specifically to the comparison drawn up in the last two columns of each of the original tables. Take for example¹ Table 1, which we have reproduced, in order to facilitate a ready check on what follows. We have designated the columns serially from left to right (1-4), and below each column we give its symbolical equivalent, where S , b , and t have the same significance as before. Column 3 as stated in the original heading, represents the *amount of heat produced per bacterium*, $\left(\frac{S}{b}\right)$, these values having been computed from the data given in columns 1 and 2. The values in column 4, however, which are compared in the text with those in column 3 have been obtained by calculation from equation (3) and thus represent the *rate of heat production per bacterium*.

* The original paper gives M (capital), apparently an error in proof-reading. By actual check m is the only quantity that can possibly be intended here. There is no indication for a change in symbols, especially one not already defined.

¹ Similar remarks apply to the experiments given in Tables 2, 3 and 4 of the original paper.

They do not represent, as the original column heading erroneously states, "Gram calories per Bacteria."² All of this is rather confusing inasmuch as we are led to believe from the original definition of m , that we shall meet with values representing *heat per bacterium per unit of time*. Having satisfied ourselves, how-

TABLE 1*

	1	2	3	4
TIME	TOTAL HEAT	TOTAL NUMBER BACTERIA $\times 10^6$	GRAM CALORIES PER BACTERIUM OBSERVED $\times 10^{-9}$	"GRAM CALORIES PER BACTERIUM CALCULATED" $\times 10^{-9}$
<i>hours</i>	<i>gram Cal.</i>			
0	0.000	360	0.000	0.000†
1	1.485	420	3.540	4.608‡
2	17.680	2,160	8.182	8.770
3	50.290	9,600	5.240	5.950
4	76.650	33,600	2.280	2.650
5	92.730	96,000	0.966	1.080
6	108.600	129,000	0.842	0.829
Symbolic equivalent...	S	b	$\frac{S}{b}$	$\frac{S}{\int_0^t \frac{t}{2^t} dt}$

* Recompiled from table 1 of the original paper (1).

† Strictly taken this value is indeterminate and not equal to 0. See equation (3).

‡ Our result = 3.77×10^{-9} . For explanation see footnote 3.

ever, by actual check that the values in column 4 have been calculated by means of equation (3) we see that this column accordingly gives,³

$$\frac{S}{B \int_0^t \frac{t}{2^t} dt}$$

² Accurately quoted.

³ We have been unable to check the result given for the 1st hour, that is, 4.608. Our result is:

$$\frac{1.485 \times 2.303 \log_{10} \frac{420}{360}}{1 \times 60 \times 10^6} = 3.77 \times 10^{-9}$$

But, in spite of what has been said, it will be noted that there is a remarkable parallelism between the corresponding values in these two columns. This leads to still further confusion unless we keep clearly in mind what is actually being represented and compared. Granting, for the moment, that the foregoing correspondence is real, let us take the matter one step forward. Since the numerical agreement between these columns is of a high order,—differences being attributable to experimental errors, we may equate the symbolical equivalents of these two columns, and we have,

$$\frac{S}{b} = \frac{S}{B \int_0^t 2^{\theta} dt}, \quad (4)$$

That is to say,

$$b = B \int_0^t 2^{\theta} dt.$$

But from equation (1)

$$b = B 2^{\frac{t}{2}}.$$

Hence,

$$B 2^{\frac{t}{2}} = B \int_0^t 2^{\theta} dt! \quad (5)$$

Evaluating the integral as before, cancelling B on both sides,

Compare, however, the result for the 2nd hour:

$$\frac{17.68 \times 2.303 \log_{10} \frac{2160}{360}}{2 \times 1800 \times 10^6} = 8.77 \times 10^{-9}$$

a value which checks with that given in the authors' table. Note that the value of B has to be taken as 360×10^6 in order to verify the authors' results. To do this neglects the fact that a slight but definite lag period preceded strictly logarithmic growth in this experiment.

collecting and rearranging terms, and finally dividing by $g2^{\frac{t}{\sigma}}$ we get,

$$\frac{\log_e 2}{g} = 1 - 2^{-\frac{t}{\sigma}} \quad (6)$$

Now,

$$\frac{\log_e 2}{g} = \text{constant.}$$

But, the right hand side of (6) is not constant since it contains a function of t . This result proves beyond all question that the relation expressed in equations (4) and (5) is untrue, and we are, therefore, obliged to regard the comparison between columns 3 and 4 as illegitimate.

Our position now is about this: Critical examination of the text and tables has amply demonstrated that the comparisons just discussed are unsound, for it is perfectly clear that *heat production per bacterium* and *rate of heat production per bacterium* are two entirely different things, any numerical correspondence between them to the contrary. Furthermore, even if we should accept the tabulated agreement as significant, we could not escape the paradoxical conclusion that a variable is constant. This paradox is, of course, merely a necessary consequence of the fact already indicated, that the authors have in reality compared units of unlike dimensions.

At first glance it might appear possible to correct this error by dividing the values in column 3, which represent $\left(\frac{S}{b}\right)$, by t , thus obtaining a new set of "observed" values which would then be expressed in the same unit as that of column 4, namely, rate of heat production per bacterium. Actually, however, we should thereby not only destroy the numerical agreement heretofore existing between "experimental" and "theoretical" determinations, but we should also be making an obviously fruitless effort in trying to compare $\left(\frac{S}{bt}\right)$ "observed" with $\left(\int_0^t bdt\right)$ as given by equation (2).

The difficulty here depends chiefly upon the fact that the values obtained from $\left(\frac{S}{bt}\right)$, although expressed in the proper unit, constitute, as long as t is finite, only a very unsatisfactory approximation to the results given by $\left(\frac{S}{\int_0^t bdt}\right)$.⁴ Thus, it is clear that the latter quantity cannot be taken as a measure of the accuracy in the former. Furthermore, if we are disposed to regard $\left(\frac{S}{bt}\right)$ as an "observed" value for the rate at which a single bacterium produces heat, we are likewise obliged to consider $\left(\frac{S}{\int_0^t bdt}\right)$ an "observed" rather than a "theoretical" or "calculated" value of m , since the elements entering into each of these expressions are as truly a matter of observation in the one case as in the other, the terms in the numerator even being identical. It is, therefore, apparent that we are not dealing in this particular instance with "observed" and "calculated" values at all, for this distinction applies only to results that have been independently obtained. Indeed, such a comparison is equivalent in many respects to an attempt at identifying a sharply-defined photograph by another of the same view which has been taken more or less out of focus. It would have been more acceptable, for instance, to have compared theoretical with observed values for S , since heat had actually been measured calorimetrically on the one hand, and could be calculated from the growth curves by equation (3) on the other, provided, of course, that m had been known rather than sought.

To sum up, we have now seen that the comparisons which the authors have made are clearly in error, and we have also seen that the comparisons which they apparently intended to make

⁴ Proof: Evaluate the integral in the denominator as before, expand the logarithmic term in the result on the condition that $\frac{b}{B} > 1/2$, and neglect quantities of an order higher than the first. This gives, $m = \frac{S}{bt}$.

do not serve their original purpose. Accordingly, it can readily be understood why we have found it necessary to disagree with the statement that "the use of this equation is of great assistance in checking results and in reaching a decision as to the significance of unexpected values." As a matter of fact, if S as well as b and B are subject to direct measurement, we prefer to look upon the formula merely as the method by which m can be precisely determined whenever heat is liberated during the logarithmic period in proportion to the number of organisms then inhabiting the culture medium.

We may now return to complete our inquiry into the significance of equations (2) and (3). Broadly speaking, it is almost certainly true that the heat output in bacterial cultures is a function of their population, and we should, therefore, expect that variations in the liberation of heat would correspond more or less accurately to changes in the number of living organisms. The authors themselves have confirmed this general relationship, although the complete details have not been given since they have described the elimination of heat simply during the logarithmic phase of growth, in which it is not unreasonable to suppose the great majority of organisms living. Under these conditions, a correlation between total heat and total number of bacteria is not open to serious question. But the matter of distinct importance which here concerns us, is the precise nature of the mathematical relation between heat and bacterial growth. Bayne-Jones and Rhees, as we have seen, have assumed that each bacterium liberates heat at a constant rate and consequently they conclude that the total amount of heat is directly proportional to the number of organisms present in the culture medium. They have then employed the Buchanan formula, which has been derived on such assumptions, as an indirect method of calculating the unit rate of heat production, (which they cannot or at least have not observed) intending thereby to get additional information concerning the processes involved. In this connection we have already called attention to the fact that, however reasonable the Buchanan formula may appear, there is nothing to prove that it represents a necessarily true relation between

heat production and bacterial growth. We have thus come to the point where we desire to bring forward the evidence upon which we base our own conclusion that the liberation of heat in the experiments under discussion has not been, and furthermore, cannot be, correctly expressed by equation (3).

In the first place, if observation and theory are on common ground, we must expect that the numerical values obtained from the right hand side of (3) will give a constant, or approximately constant value for m , since the rate of heat production per bacterium has previously been supposed "definitely uniform." An inspection of the tables and curves in the original paper, however, (we again refer to table 1 as an example), shows that the rate of heat production of a single bacterium has apparently been found to vary throughout the logarithmic period of growth,⁵ rising from zero to a maximum *during* (not at the end of) the second hour, and thereupon declining gradually to the end of the experiment.⁶ But it is perfectly clear that the foregoing results cannot be reconciled with the fact that the unit rate of heat production has not only been assumed constant, but, in addition, has actually been treated so. Indeed, we are in turn, inclined to regard this "variation" in the rate of heat production as *prima facie* evidence that bacteria do not liberate heat in accordance with the Buchanan formula, much as a change in the velocity constant of an apparently first order chemical reaction is interpreted to mean that the reaction fails to obey the monomolecular law. Nevertheless, in order to place this matter beyond any question whatsoever, it will be profitable, we believe, to consider the problem from another point of view.

To begin with, careful inspection of the original curves shows that heat output rises gradually for the first hour and then becomes almost exactly linear for a period of three to four hours

⁵ Even if this should prove to be true, it would not signify that "young bacterial cells produce more heat per cell than older ones."

⁶ We are unable to agree that "a period of greatly reduced metabolic activity on the part of each bacterium begins at about the fourth or fifth hour and is maintained at a low constant rate for ten hours, and probably for a longer time," nor are we convinced that "the curves in the illustrations in this paper clearly show this prolonged constant heat production per cell."

or even longer. During this same period bacterial growth is practically logarithmic save for a slight but definite lag phase confined entirely to the first hour in the two experiments illustrated in figures 1 and 3 of the authors' paper. This linear relationship between heat output and time we especially desire to emphasize, because we are thereby obliged to conclude that the relation between these variables may reasonably be expressed, for the above interval in the form,

$$\frac{dS}{dt} = \text{constant.} \quad (7)$$

The simplicity of equation (7) merely implies that we are neglecting differences of the second and higher orders, since we are certainly not in a position, on the basis of the experimental data alone, to infer a more complicated, not to mention a more correct, expression for the rate of heat elimination. Viewed as a matter of observation, then, we may accept the rate of heat production as sensibly constant. But, when we turn, on the other hand, to examine the nature of the theoretical curve of heat production we find, in contradistinction to the foregoing result, that the rate at which heat has been assumed to be liberated is by no means constant, for differentiating (3) with respect to t , after substituting $B2^{\frac{t}{g}}$ for b and cancelling like terms, gives,

$$\frac{dS}{dt} = mB2^{\frac{t}{g}}, \quad (8)$$

an expression, as we may recall, from which the definite integral in equation (2) has been set up. According to (8), the rate of elimination must increase in geometric ratio with time, and it is, therefore, clear, that this equation cannot represent the phenomena to which the previous equation, (7) applies.

An effective demonstration of the foregoing results is given by the material here set up in table 2 and by the curves in figure 1, for which we have again used the data presented in the first table of the original paper. The observed values for S , taken from the third column of our table are nicely distributed about the

broken line in the lower portion of the figure. The theoretical values for S , on the other hand, lie upon the smooth curve that rises far too rapidly even within this relatively short 4 hour period. The latter values have been computed as follows:

1. With the aid of equation (1) in its logarithmic form,

$$\log_{10} b = \log_{10} B + \frac{t}{g} \log_{10} 2, \quad (9)$$

we have calculated the mean values of B and g from the data

TABLE 2

TIME	NUMBER OF BACTERIA CALCULATED FROM EQUATION (9) $\times 10^6$	$m \times 10^{-9}$	HEAT OUTPUT (S)	
			Observed in experiment 1	Calculated from equation (3) when $m = 7.9 \times 10^{-9}$
hours			gram Cal.	gram Cal.
0	105.4	—*	0.000	0.00
1	457.0	6.15	1.485	1.90
2	1,990.0	13.75	17.680	10.15
3	8,640.0	8.64	50.290	46.00
4	37,400.0	3.02	76.650	200.50

* Indeterminate.

by the method of averages over the four hour period manifesting geometric growth, and have found,

$$\begin{aligned} \log_{10} B &= 8.0228 \\ B &= 105,400,000 \text{ organisms} \\ g &= 0.471 \text{ hour} \end{aligned}$$

2. We have then computed theoretical values of b indirectly from (9) for substitution into equation (3); these lie upon the straight line shown in the upper portion of figure 1. It will be noted that the initially observed value of $\log_{10} B$ is situated at a considerable distance from this curve, a fact which shows that lag, though of short duration, would seriously interfere with the subsequent calculation of S .⁷ We prefer, for the purpose of illustration, therefore, to use the value given above, since equa-

⁷ Compare footnote 3.

tion (3) provides only for strictly logarithmic growth. The remaining values are, as we see, fully in accord with this provision.

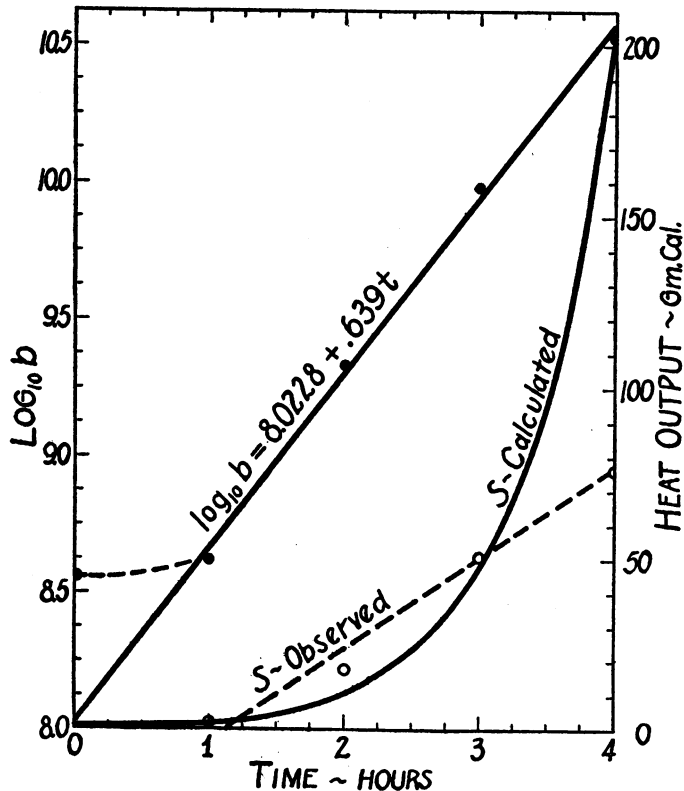


Fig. 1. The upper curve represents equation (9) fitted to the data for the first 4 hours, where $\frac{\log_{10} 2}{g} = 0.639$, and $\log_{10} B = 8.0228$. Note the presence of lag during the first hour.

The two lower curves clearly show the difference between total heat as measured experimentally and as expected according to the conditions defined by equation (3).

3. The foregoing values of b and B , with the corresponding values of t have then been substituted into equation (3) and the series of values for m determined, which are given in the second column of table 2. These, we note, do not agree numerically with similar values which the authors have obtained because of

the difference in the method of calculation to which we have just now referred. The average value for m is, 7.9×10^{-9} gm. calories per hour.

4. Upon resubstituting this value of m , as well as the above values of b , B and t into equation (3) we have finally calculated the theoretical values of S set up in column 4 of table 2 and lying, as we have already explained, upon the smooth curve in figure 1.

This curve rises somewhat too slowly at first, but during the last hour of a period that is very short indeed it shows such a tremendous increase that the final values at the end of the fourth hour differ from the observed heat output by approximately 200 per cent! The trend and the slope of the two curves clearly illustrate an increasingly conspicuous disagreement between theory and fact, in that the slope of the curve for the observed values is practically constant, whereas the slope of the theoretical curve can easily be seen to respond to a powerfully augmented function of time quite in accord with the provision of equation (8).

Thus, we are again brought to the conclusion that Buchanan's formula does not apply to the data under discussion. Moreover, since this equation fails to hold for S , it cannot be employed to calculate m . Consequently, if m is desired we need to proceed differently. Combining equation (1) with equation (7) we have,

$$m = \frac{1}{b} \frac{dS}{dt} = \frac{\alpha}{B} 2^{-\frac{t}{\sigma}} \quad (10)$$

in which α represents the value of the constant in (7). This is an important result since it proves that the curve of the unit rate of heat production is a simple diminishing exponential function of time as long as growth is logarithmic and heat output linear; it also shows that under these conditions, m does not possess either "critical" or maximal values. We must, therefore, distinguish clearly between m as given by equation (10) and the quantity $\frac{S}{b}$ which may be obtained from the relation

$$\frac{S}{b} = \frac{\alpha t}{B 2^{\frac{t}{\sigma}}} \quad (11)$$

in which the numerator of the right hand side represents the integral of (7) with the constant of integration set equal to 0, since the initial conditions provide that $S = 0$ when $t = 0$. Differentiating (11) once with respect to t and equating to 0, we have,

$$t = \frac{g}{\log_2 2} \quad (12)$$

A second differentiation gives,

$$\frac{d^2 \left(\frac{S}{b} \right)}{dt^2} = \frac{\alpha \log_2 2}{Bg} \cdot 2^{-\frac{t}{g}} \left(\frac{t \log_2 2}{g} - 2 \right). \quad (13)$$

Upon substituting the value of t from (12) into (13) we have

$$\frac{d^2 \left(\frac{S}{b} \right)}{dt^2} = - \frac{\alpha \log_2 2}{Bg} 2^{-\frac{1}{\log_2 2}} = - \frac{\alpha \log_2 2}{Bg \epsilon} \quad (14)$$

where ϵ is the Napierian base of logarithms.

Hence, since $\frac{d \left(\frac{S}{b} \right)}{dt} = 0$ and $\frac{d^2 \left(\frac{S}{b} \right)}{dt^2} < 0$ when $t = \frac{g}{\log_2 2}$, we may conclude that the curve for $\left(\frac{S}{b} \right)$ in contrast to the curve for m has a maximum, which is reached in the present example, .681 hours after the liberation of heat has begun.⁸ Again, setting (13) equal to 0 we find,

$$t = \frac{2g}{\log_2 2} \quad (15)$$

Therefore, the point of inflexion of (11) is situated twice as far away from the onset of heat production as the maximum. It

⁸ In the example already discussed heat did not begin to be liberated until the end of the first hour. The maximum, therefore, is situated at about 1.68 hours or slightly before, and the point of inflexion at 2.36 hours.

will also be noted from (12) and (15) that the position of the maximum as well as that of the point of inflexion are independent of the rate at which heat is liberated, and vary only with the generation time of the culture. The more rapidly the organisms grow, the sooner the maximum and the point of inflexion are reached.

Thus, in reviewing our evidence as a whole, we may finally conclude that the Buchanan formula cannot be employed to calculate the unit rate of heat production in the experiments which Bayne-Jones and Rhee have performed. Support for this conclusion rests chiefly upon the demonstration that heat has not been liberated in accordance with the assumptions to which this formula may be traced. Incidentally, it has become evident that the unit rate of heat production is actually a diminishing exponential function of time whenever heat output during the logarithmic phase of growth is linear, two conditions, we may recall, that have been quite satisfactorily reproduced in the experiments to which we refer. This quantity neither remains constant, as the Buchanan formula demands, nor does it vary in the manner shown to be characteristic of the curve describing unit heat production. What bearing these facts may have upon our ultimate concepts in regard to the processes concerned with bacterial metabolism, we prefer to leave to further experimental investigation.

REFERENCE

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