THE ENERGETICS OF HETEROTROPHIC BACTERIA[1]

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INTRODUCTION

D'Arcy Thompson has said, "When science wishes to speak plainly, she uses the language of mathematics." Without denying the truth of this statement, biologists in general have been skeptical of the practicability of the application of mathematics to the chemistry of the cell. The first attempts to apply the theory and practice of thermodynamics to cell phenomena met with considerable opposition; proponents of a vitalistic philosophy were loud in argument and long in speech with regard to the fallaciousness of the application of knowledge gained in experimental mechanics and thermochemistry to life processes.

The researches of Lavoisier and Laplace, Dulong and Depretz, Berthelot and Rubner definitely established that the first law of thermodynamics—the law of the conservation of energy—was fulfilled in the realm of biology as well as physics. The conception of the cell as a machine capable of transforming chemical energy into work and heat soon followed. But a necessary consequence of this conception postulated that the second law—the entropy of an isolated reacting system increases, the free energy decreases—also was satisfied by the organism. The latter was not so easy to demonstrate; many still thought that a special form of energy characteristic of protoplasm was concerned in the energy transformations of the cell. This "vital energy" did not necessarily obey the laws of classical thermodynamics. Apparent examples of departure from the second law exhibited by colloidal solutions strengthened this view, and it was even

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proposed that bacteria and other microörganisms might serve as "Maxwell demons" in the transfer of energy to a higher potential without work being supplied from a source outside the system. Because of these and other philosophical implications, the question of the validity of the second law in the realm of biology has engaged the attention of numerous writers. For reviews of this aspect of the subject the reader is referred to the papers of Meyerhof (48), Höber (29), Wachtel (69), Oppenheimer (53) and Guye (23).

The idea that the second law of thermodynamics was of a statistical nature and that entropy was a measure of the probability of a displaced equilibrium readjusting itself did much to elucidate the difficulties attendant on the anomalous behavior of microscopic bodies. The statistical nature of the law admits exceptions in a small population, but if the numbers approach those attained in gas volumes or in bacterial cultures, these deviations are eliminated as far as the gross effects are concerned. A discussion of the possible limitation of thermodynamical treatment of minute particles from the point of view of probabilities is given by Donnan (17) and Guye (23).

However certain we may be concerning the validity of the laws of thermodynamics in biological processes, the study of the energy relations of the cell offers experimental difficulties that to date have defied investigations other than those that deal with mass changes. As Linhart (41) has pointed out, the biologist in presenting his problems to the physical chemist is usually met with the statement that "biological processes are so highly complex that they are not susceptible to thermodynamic treatment." In a consideration of the cell as a machine operating in an isothermal manner to perform work, to liberate heat, and to synthesize substances of high energy content from materials of low energy value, it soon becomes obvious that complicated reactions occur and these reactions are coupled in a manner that is completely absent in the relatively simple, isolated systems usually studied in thermochemistry. However discouraging the outlook may be, some progress, partly of an experimental and partly of a theoretical nature, has been made.

The more general aspects of the energetics of the cell have been considered in the texts of Meyerhof (48), Oppenheimer (53), and Höber (29). Also Kruse (36) devotes part of a chapter of his "Allgemeine Mikrobiologie" to a treatment of the energetics of microörganisms, and the more recent texts of Buchanan and Fulmer (9) and Stephenson (63) discuss certain aspects of this field of bacteriology. Bacteria have been conveniently separated into two classes as regards the source of their energy and its utilization—the autotrophic and the heterotrophic. The former obtain their energy by oxidation of ammonia, nitrites, sulfur, etc., and perform true thermodynamical work in the synthesis of carbohydrate material from carbon dioxide. The heterotrophs, which comprise by far the majority of the known organisms, obtain their energy by oxidation or dissimilation of organic matter. Because of the relative simplicity of the processes involved in the metabolism of the autotrophs, much more attention has been given to the energetics of these organisms than has been devoted to the energy changes of the more complex decompositions brought about by the heterotrophic forms. The energetics of the first group have been adequately discussed by Meyerhof (43), Waksman (70), Buchanan and Fulmer (9), and Baas-Becking and Parks (2). This review will deal only with the second group, and to some extent with the yeasts, since the metabolism of the latter is very similar to that of many of the bacteria considered.

For convenience, this discussion is divided into three parts: (*1*) the general problem of energetics, which deals with the methods of study and with the source and possible utilizations of the energy available to the organism; (*2*) the application of energetics to typical fermentation processes; (*3*) the energetics of nitrogen fixation by bacteria.

I. GENERAL PROBLEM OF ENERGETICS IN BACTERIOLOGY

Methods used

Thermochemistry. In the field of bacterial energetics, the pioneer work of Rubner pointed the way in methods as well as in

the interpretation of results. In his first studies (57, 59), he made use of what might be termed "differential calorimetry" to gain an idea of the energy changes taking place in the growth of bacteria. The method, laborious and subject to numerous errors, was simply the determination of the calorific value of the medium before and after growth of bacteria. From the difference he calculated the energy necessary for the maintenance of the cell. The bacteria were removed by precipitation with iron, and the calorific value of the cells was estimated. From these data

TABLE 1

Energy changes produced by growth of certain bacteria

Rubner

ORGANISM	INCREASE IN BACTERIAL NITROGEN	LOSS OF ENERGY BY MEDIUM	ENERGY VALUE OF BACTERIAL CELLS		ENERGY OF MAINTENANCE PER GRAM BACTERIAL NITROGEN PER DAY
	milligrams	*kilogram-calories*	*kilogram-calories*	*per cent**	*kilogram-calories*
B. pyocyaneus	190	24.70	6.84	27.7	15.6
B. coli	69	7.86	2.42	30.8	18.1
B. proteus	105	15.58	3.26	20.9	19.4
B. thermophilus	79	18.15	4.68	24.9	34.5
B. typhosus	42	11.18	1.30	11.6	42.8
V. cholerae	61	19.69	3.35	17.0	42.7
B: diphtheriae	35	13.48	1.63	12.1	60.6

* Percentage of energy lost by medium.

he could estimate the percentage of total energy that "disappeared" which reappeared in the cellular crop. Naturally this method was limited to a study of those organisms that did not produce gases (other than carbon dioxide) in their metabolism. Table 1 taken from his data gives typical results.

It will be noticed that those organisms which did not grow so well had high values for the energy of maintenance and low values for the percentage of the total energy changed that appeared in the organisms. The same medium (6 per cent beef extract) was used for all the organisms. It is probable that it was not suited to the physiology of some of the bacteria, and as a

consequence a higher expenditure of energy per unit weight resulted than is actually necessary for the functioning of that particular organism. A repetition of the experiment employing the medium best suited to the individual organism might show entirely different results.

It is of interest to compare the values obtained for bacteria with those of other species investigated by Rubner. The values are shown in table 2. Rubner attributed the lower values for the more complex organisms to the fact that by reason of their organization the liberated energy is used more economically.

TABLE 2

Energy of maintenance of various species

Rubner

SPECIES	ENERGY OF MAINTENANCE PER GRAM NITROGEN PER DAY
	kilogram-calories
Man	1.0
Dog	3.28
Mouse	15.19
Sparrow	17.4
Cold-blooded animals	0.4–0.6
Yeast	38.77
Bacteria	15.6–60.6

Using *B. proteus* as the experimental organism, Rubner (59) investigated the influence of temperature, age, and other variables on the energy changes. Temperature changes from 14°C. to 36°C. made little difference in the total energy change, but the rate was somewhat slower at the lower temperature. In the earlier stages of the fermentation (1 to 10 days), the total energy expended was two to three times larger than that during the later stages. Rubner attributed this to the difference of the energy requirement of the organism during the growth phase and during the "resting" period.

Tangl (65) also studied bacterial energetics as part of the problem of the energetics of ontogenesis, i.e., the energy changes coincident with the development of an individual. He used the

method of "differential" calorimetry and estimated the energy expended in the synthesis of 1 gram of dry bacterial substance. The results of one experiment gave the following values: *B. anthracis*, 8.6 kg-cal.; *B. suipestifer*, 16.5 kg-cal.; *B. subtilis*, 12.5 kg-cal. The potential energy of the nutrients was 4.3 kg-cal. per gram and that of the cells 4.5 kg-cal.; yet in the various reactions that accompanied the synthesis of 1 gram of cell protoplasm, there disappeared from the medium nutrients equivalent to from 8.6 kg-cal. to 16.5 kg-cal. Tangl believed that little of this energy was actually used in cell synthesis.

Calorimetry. Due to the limitation of the differential calorimetric method, Rubner (59) developed a technique for following energy changes during growth of organisms by measuring the actual heat produced. He constructed a "bio-microcalorimeter" that would detect energy changes of the order of one to two gram-calories and followed the heat production of pure and mixed bacterial cultures on a variety of media. With *B. coli* under anaerobic conditions, he found that 68 per cent of the total energy expended appeared as heat and with *B. proteus* only 15 to 25 per cent. Unfortunately, the data for the total energy used were not from the same experiment in which the heat production was measured, hence these figures can be regarded only as approximate, and are very likely too low. Since Rubner's work, the technique of measuring heat production by means of the calorimeter has been greatly improved and a check on some of his findings would appear to be desirable. Hill (27) has developed a very sensitive differential microcalorimeter in which heat production is measured by a thermocouple instead of a Beckmann thermometer. By the use of this calorimeter, Shearer (62) showed that the heat production of *B. coli* was seven to eight times as great in a glucose-peptone broth as in a broth of the tryptic digest of casein, and that the heat produced by *B. coli* in casein digests was inversely proportional to the length of digestion. He attributed these results to a more economical utilization of energy by *B. coli* in the presence of amino acids. Unfortunately, no data are given for the heat production in glucose-tryptic digest of casein broth, hence the influence of the glucose on the economy was not

determined. Bayne-Jones (3, 4) has recently described a differential microcalorimeter of the Hill type especially adapted to bacterial fermentations and has studied heat production by *B. coli* and *Staph. aureus* during the various growth phases. The data presented indicated that during the logarithmic growth phase of *B. coli*, the heat production was a linear function of the time. As pointed out by Wetzel (72), this suggests that the rate of heat production per cell is not constant but diminishes exponentially with time. Further contributions concerned with heat production in other growth phases are necessary before a satisfactory interpretation of the significance of this observation can safely be made.

Meyerhof (44) studied heat production of bacteria in comparison with oxygen uptake. The heat produced was measured in a microcalorimeter in which 0.01° rise on the Beckmann thermometer corresponded to 2.5 g-cal. Parallel determinations of the oxygen uptake were made on the same culture in a Warburg microrespirometer or by Winkler's titration method. The quotient $\frac{\text{g-cal. produced}}{\text{mg. } O_2 \text{ consumed}}$ was used as a measure of the metabolism. Meyerhof states this should be 3.2 for protein, 3.4 to 3.5 for carbohydrate, and 3.3 for fat. He grew *Vibrio Metchnikoff* in a medium that was not suited to the physiology of the organism, and found this quotient to be 3.95 with an error of 10 to 15 per cent. In a medium that allowed good growth, this quotient rose in one case to 4.1, and in another medium to 4.5. Meyerhof concluded that energy-liberating processes other than oxidations took place in this organism, since with blood cells, sea urchin eggs, etc., the quotient was constant irrespective of whether growth was retarded or not. Dissimilation processes, neutralization of acids and heat of solution of metabolic products might account for some of the excess energy.

Thermodynamics. Aside from the practical methods for the estimation of energy changes of the cell, a method is available that is more theoretical in character. This is an application of thermodynamics to the cell processes. Although there are obvious limitations to this method, and its free use of mathematics

discourages many biologists, it offers a tool for investigation that should not be disregarded. As long as the proper skepticism is maintained toward any data which cannot be directly verified in the laboratory, the danger that arises from the use of thermodynamics or any other theoretical aid is often far outweighed by the information given. For example, the judicious use of thermodynamics will often prove of value in the test of proposed mechanisms for unknown processes, and while thermodynamics is not concerned with any particular mechanism, its use may help to decide the most probable one. If a mechanism for a given biological process is known, the use of thermodynamics enables the experimenter to gain some idea of the energy available for work that arises from the process. For some time these advantages have been utilized by bacteriologists in a manner that is inaccurate and which takes no cognizance of the fact that the concentration of the components of a reaction affects the quantity of available energy liberated. It is a common practice to determine the metabolic products of an organism, to construct equations for the chemical changes involved, and, from thermochemical data (heats of combustion and formation), to determine the energy "available" to the organism. This procedure is often only a rough approximation, as the following discussion will show.

It is not in the scope of this paper to detail the chemistry and mathematics involved in the subject of thermodynamics as applied to chemical reactions. For a thorough presentation of the subject, Lewis and Randall's text (38) may be consulted, but for the biologist who wishes to avoid the more detailed application, the briefer treatment of the material by Buchanan and Fulmer (9) and by Fulmer and Leifson (19) is probably sufficient. However, two fundamental equations with their meaning should be considered here. In the calculation of energy changes prior to the Nernst (51), or the Lewis and Randall systems of thermodynamics, the criterion of the driving force of a reaction was the heat of reaction ($-\Delta H$). However, these authorities, following Gibbs, have shown that the change in free energy ($-\Delta F$) and not the heat of reaction ($-\Delta H$) is the true criterion. Free energy is the quantity of energy liberated in a reaction that is

available for doing work. $-\Delta F$ may be greater or less than $-\Delta H$ depending on the temperature coefficient of the former. If this temperature coefficient is negative, the free energy is less than the heat of reaction and *vice versa*. In the former case, the energy not available for work $T\left(\frac{\partial \Delta F}{\partial T}\right)_P$ is that part of the energy that must disappear as heat in order that the reaction may proceed. In case $-\Delta F$ is greater than $-\Delta H$, the excess energy is taken from the surroundings as heat and converted into a form capable of the performance of work. Hill (28) has emphasized the importance of the concept of free energy in biology and has discussed a few reactions which are endothermic if the heat of reaction is used as a criterion, but which can be used to do work on account of the conversion into free energy of heat from the environment. The relations between ΔF and ΔH are given by the following equations:

$$\Delta F = \Delta H - T\,\Delta S$$

or

$$\Delta F = \Delta H + T\left(\frac{\partial\, \Delta F}{\partial T}\right)_P$$

where ΔS is the change in entropy, T is the absolute temperature, and $\left(\frac{\partial \Delta F}{\partial T}\right)_P$ is the temperature coefficient of free energy at constant pressure. By reason of certain conventions of signs, a reaction is said to occur spontaneously, i.e., with liberation of free energy, when ΔF is negative. It must be recognized that $-\Delta F$ as defined above is the maximum energy available for work, and the actual work done will depend on the efficiency of the process involved. For a strictly reversible system, e.g., a galvanic cell nicely balanced, an efficiency of 100 per cent is possible, but in more practical applications such as the storage battery the efficiency is less and part of the free energy disappears as heat.

A second factor, which is of the utmost importance to the biologist, but which is often overlooked, is the change in $-\Delta F$ with changes in concentrations, or more accurately with activities

of the reactants and resultants. The value of $-\Delta F$ for a particular substance given in the literature is usually that for the element or compound when present in the standard state. The standard state is defined as the state in which the activity of the substance is unity. Thus at 25°C. and under 1 atm. pressure solid glucose, liquid water, gaseous oxygen and a molal solution of a solute (with proper correction for deviations from a perfect solution) are in the standard state. If a substance is transferred from its standard state to some other state, as by change of temperature, pressure, or concentration, energy changes are involved. It follows that if the reactants or resultants of a given reaction are present in a state other than the standard, the calculated free energy change must be corrected, since the calculation is based on the reaction in which each component appears in the standard state. The correction is given by the equation

$$\Delta F = \Delta F^\circ + RT \ln K,$$

where ΔF is the increase in free energy under the conditions studied, ΔF° equals the increase in standard free energy, R is the gas constant, T is the absolute temperature, and K is the equilibrium constant of the reaction. An example taken from Burk (11), dealing with fixation of nitrogen by legumes, may serve to make this clear.

$$1/2\ N_2\ (g) + 5/4\ O_2\ (g) + 1/2\ H_2O\ (l) \rightarrow H^+ + NO_3^-$$
$$-\Delta F^{\circ *} = 1/2\ (0) + 5/4\ (0) + 1/2\ (+56{,}560) \rightarrow (+26{,}500)$$
$$= -28{,}280 + 26{,}500 = -1780\ \text{g-cal.}$$

* In this paper ΔF° refers in all cases to ΔF° at 25°C.

But if this reaction takes place at the concentrations that are likely to exist in the plant (legume), a different result is obtained, viz.: $HNO_3 = 0.0001\ M.$, $O_2 = 0.2$ atm., and $N_2 = 0.8$ atm.

$$\therefore K = \frac{(0.0001)^2}{(0.8)^{1/2}\ (0.2)^{5/4}} = 8.6 \times 10^{-8}$$

$$\log K = -7.07$$

$$-\Delta F = -1780 + 1365\ (-7.07) = 7870\ \text{g-cal.}$$

In this case it is observed that owing to change in concentrations (and hence activities) the sign of $-\Delta F$ is changed, and whereas the reaction would require energy from an outside source if each substance is present in its standard state, it will proceed spontaneously when the concentrations are changed.

In conclusion it must be noted that for precise work K must be calculated from the activities of the substances involved. However, concentrations may be used for this calculation in most of the ranges found in biological studies, where because of the complexity of the system, activities are often difficult to determine.

Baron and Polányi (5) were among the first workers to apply thermodynamics to biological processes. They made use of the Nernst heat theorem (51), which is not quite as accurate as the more recently developed system of Lewis and Randall. Considering animals in which the energy changes were due to the combustion of protein, fat, or carbohydrate, to the change of carbohydrate into fat, and to the production of work, Baron and Polányi calculated the free energy changes of these processes and made a balance which showed that the change in free energy could be positive only when heat is absorbed from the environment. In homiotherms the opposite takes place, consequently the total metabolism of these species results in a decrease in free energy. This means that at least in this case of biological energetics the second law of thermodynamics is valid. Experiments with poikotherms, e.g., bacteria, show that their total metabolism always results in a liberation of heat. Therefore it appears that the conclusion of these authors is not limited to homiotherms. In the calculations of Baron and Polányi, $-\Delta A$ was used instead of $-\Delta F$. The former is the free energy decrease at constant volume and differs from $-\Delta F$ in the same way that $-\Delta V$ differs from $-\Delta H$, viz., the PdV factor is not included.

Utilization of energy

The results of Rubner and Tangl showed that only 15 to 60 per cent of the energy that disappeared from a medium in which bacteria had grown appeared in the cells. Since the work of these investigators, similar results have been obtained for a large number of organisms, some of which are given in table 3.

These figures indicate that during the synthesis of 1 gram of bacterial substance (the equivalent of 4.5 kg-cal.), there is liberated from two to over one hundred and twenty times this quantity of energy. The question naturally arises—of what use is this energy to the bacteria? A few possible expenditures will be taken up in an effort to account for the energy which is apparently at the disposal of the organism.

Energy of synthesis. The compounds synthesized by bacteria and built into their cells include many types found in both plants and animals (9). Carbohydrates of various types have been isolated—polysaccharides, gums, and capsular substances, as well

TABLE 3

Energy of medium transformed into bacterial cells

ORGANISM	MEDIUM	PERCENTAGE OF ENERGY CHANGE REPRESENTED BY BACTERIA
B. pyocyaneus	Asparagin	19.0
Lactic acid bacteria	Peptone-milk	46.0
Microc. ureae liquefaciens	Urine + urea	0.8–32.0
Bac. aerogenes	Whey + lactose and peptone	6.5–30.0
Acetic acid bacteria	Alcohol	0.75
Yeast	Wort	22.0–43.0
Mannitol formers	Glucose	48.0
Azotobacter	Mannitol	5.0–11.0

as reserve materials such as glycogen and granulose. Fatty materials of even greater variety and complexity are known to occur in bacterial cells. These include free fatty acids, glycerides, waxes, sterols, and phosphatides. Simple proteins, as albumin and globulin, have been obtained from bacteria, but the major protein constituents are apparently conjugated proteins such as nucleoproteins.

It is obvious that little can be done toward deciding the question as to the energy requirements for synthesis unless we consider specific reactions. For example, the synthesis of bacterial protoplasm from a mixture of amino acids and polypeptides,

such as beef extract, can be done with much less expenditure of energy than is necessary if the building material consists of glucose and nitrates and other salts. In the latter case energy is required to reduce the nitrate to ammonia and to combine this with decomposition products of glucose to form the various amino acids required for the proteins of the organism. This necessary energy is furnished by an oxidation of part of the glucose, as will be discussed in detail later. Beebe and Buxton (6) have shown that *B. pyocyaneus* can synthesize fat on a sugar-free peptone broth, the synthesis of fat from carbohydrates by yeast has long been known, and the work of Lieben (39, 40) and his associates demonstrated that yeast could form carbohydrate material from alcohol, lactic acid and pyruvic acid. A single amino acid can serve as the sole source of carbon and nitrogen for some bacteria, and syntheses of protein from glucose and nitrate, ammonia, or even free nitrogen are not uncommon. An examination of the energy changes involved in these interconversions might be of value in estimating what the energy requirements for synthesis are.

The synthesis of a fatty acid from a carbohydrate necessitates a reduction which requires energy. For example, butyric acid, formed by the reduction of glucose, would yield on complete oxidation 508.2 kg-cal. of free energy, while that given by glucose of an equivalent carbon content (2/3 mole) would be 457.3 kg-cal. The difference, -50.9 kg-cal., represents $-\Delta F$ for the synthesis of 1 mole of butyric acid from 2/3 mole of glucose; the negative sign denotes that energy must be supplied in order that the synthesis might take place. This energy requirement can be arrived at by a consideration of schematic equations which illustrate the reduction. While the following equations are not intended to represent the actual mechanism of the transformations, they serve as a convenient means of presenting the energy relations involved.

(1) Synthesis of butyric acid.

$$2\ H_2O \longrightarrow 2\ H_2 + O_2$$

$$-\Delta F^\circ = -113.1 \text{ kg-cal.}$$

$$2/3\ C_6H_{12}O_6 + 2\ H_2 \longrightarrow C_3H_7COOH + 2\ H_2O$$

$$-\Delta F^\circ = 62.2 \text{ kg-cal.}$$

$$1/3\ C_6H_{12}O_6 + 2\ O_2 \longrightarrow 2\ CO_2 + 2\ H_2O$$

$$-\Delta F^\circ = 228.6 \text{ kg-cal.}$$

$$C_6H_{12}O_6 + O_2 \longrightarrow C_3H_7COOH + 2\ CO_2 + 2\ H_2O$$

$$-\Delta F^\circ = 177.1 \text{ kg-cal.}$$

The sum of the decrease in free energy of the first two reactions, resulting in the formation of butyric acid, is -50.9 kg-cal. as already noted. This energy could be supplied by the simultaneous oxidation of glucose as in the third reaction. The excess of energy liberated in the complete reaction would be available for other energy-requiring processes or could be liberated as heat. In the foregoing equations, it was assumed that oxygen or other hydrogen acceptors are present that can oxidize any hydrogen liberated. If the hydrogen appears as the free element, the net free energy released by the final complete equation would be less by $56.56n$ kg-cal. where n equals the number of moles of hydrogen set free in the process.

(2) Synthesis of palmitic acid. The synthesis of palmitic acid requires 490 kg-cal. of free energy, as indicated by the following equations:

$$14\ H_2O \longrightarrow 14\ H_2 + 7\ O_2$$

$$-\Delta F^\circ = -791.8 \text{ kg-cal.}$$

$$8/3\ C_6H_{12}O_6 + 14\ H_2 \longrightarrow C_{15}H_{31}COOH + 14\ H_2O$$

$$-\Delta F^\circ = 301.8 \text{ kg-cal.}$$

$$4/3\ C_6H_{12}O_6 + 7\ O_2 \longrightarrow 8\ CO_2 + 6\ H_2O + 2\ H_2$$

$$-\Delta F^\circ = 801.4 \text{ kg-cal.}$$

$$4\ C_6H_{12}O_6 \longrightarrow C_{15}H_{31}COOH + 8\ CO_2 + 6\ H_2O + 2\ H_2$$

$$-\Delta F^\circ = 311.4 \text{ kg-cal.}$$

If the synthesis is carried out in the presence of oxygen or other hydrogen acceptors, the liberated hydrogen may be oxidized to water and the net decrease in free energy will be greater by 113.1 kg-cal.

(3) Synthesis of amino acids. The energy transfers that accompany the formation of an amino acid from a carbohydrate may liberate or require energy depending upon the individual amino acid, the source of nitrogen, and whether or not free hydrogen is disengaged in the reaction. If the amino acid is relatively simple and ammonia nitrogen is available, there is a release of energy.[2]

Synthesis of alanine

$$1/2\ C_6H_{12}O_6 \longrightarrow CH_3COCOOH + H_2$$

$$-\Delta H = -11.4 \text{ kg-cal.}$$

$$CH_3COCOOH + NH_4^+ + OH^- \longrightarrow CH_3CHNH_2COOH + H_2O + 1/2\ (O_2)$$

$$-\Delta H = -22.6 \text{ kg-cal.}$$

$$H_2 + 1/2\ O_2 \longrightarrow H_2O$$

$$-\Delta H = 68.4 \text{ kg-cal.}$$

$$1/2\ C_6H_{12}O_6 + NH_4^+ + OH^- \longrightarrow CH_3CHNH_2COOH + 2\ H_2O$$

$$-\Delta H = 34.4 \text{ kg-cal.}$$

However, energy is required for the synthesis of a higher amino acid, even though the source of nitrogen is already reduced, e.g., ammonia nitrogen. For example, leucine requires 97.5 kg-cal. per mole for its formation from glucose and ammonia. This energy can be furnished by the combustion of glucose, as illustrated with the fatty acids.

Synthesis of leucine

$$6\ H_2O \longrightarrow 6\ H_2 + 3\ O_2$$

$$-\Delta H = -410.3 \text{ kg-cal.}$$

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[2] In the calculation of the energy changes coincident with the formation of amino acids, it was necessary to use $-\Delta H$ as a measure of the changes taking place since free energy data on the amino acids are not available. This measure is to be regarded as a more or less close approximation of the more exact $-\Delta F$.

$$2\ C_6H_{12}O_6 + 6\ H_2 + 2\ NH_4^+ + 2\ OH^- \longrightarrow 2(CH_3)_2CHCH_2CHNH_2COOH + 10\ H_2O$$

$$-\Delta H = 215.2 \text{ kg-cal.}$$

$$1/2\ C_6H_{12}O_6 + 3\ O_2 \longrightarrow 3\ CO_2 + 3\ H_2O$$

$$-\Delta H = 337.0 \text{ kg-cal.}$$

$$2\text{-}1/2\ C_6H_{12}O_6 + 2\ NH_4^+ + 2\ OH^- \longrightarrow 2(CH_3)_2CHCH_2CHNH_2COOH + 7\ H_2O + 3\ CO_2$$

$$-\Delta H = 141.9 \text{ kg-cal.}$$

If the source of nitrogen used by the organism is in the form of a nitrate, energy must be expended to reduce this to ammonia nitrogen. In this case the formation of an amino acid as simple as alanine requires 65.9 kg-cal. of energy, which can be supplied by the oxidation of a carbohydrate.

$$H^+ + NO_3^- + 2\ H_2O \longrightarrow NH_4^+ + OH^- + 2\ (O_2)$$

$$-\Delta H = -100.3 \text{ kg-cal.}$$

$$1/2\ C_6H_{12}O_6 + NH_4^+ + OH^- \longrightarrow CH_3CHNH_2COOH + 2\ H_2O$$

$$-\Delta H = 34.4 \text{ kg-cal.}$$

$$1/2\ C_6H_{12}O_6 + 3\ O_2 \longrightarrow 3\ CO_2 + 3\ H_2O$$

$$-\Delta H = 337.0 \text{ kg-cal.}$$

$$C_6H_{12}O_6 + H^+ + NO_3^- + O_2 \longrightarrow CH_3CHNH_2COOH + 3\ H_2O + 3\ CO_2$$

$$-\Delta H = 271.1 \text{ kg-cal.}$$

There is a third way by which the needed ammonia might be obtained for the synthesis of amino acids, i.e., the hydrolysis of other amino acids. If an organism is grown in a mixture of amino acids (as beef broth, peptone) the energy value of which is very near that of the cell, the assumption is often made that no energy is required to convert the constituents of the medium into cell protoplasm. This is not necessarily the case, since it is more likely that the organism changes the substrate into other forms before using it for synthetic purposes. However, the energy

changes are not of a large order of magnitude since hydrolyses predominate among the reactions.

$$CH_3\,CHNH_2COOH + H^+ + H_2O \rightarrow CH_3CHOHCOOH + NH_4^+$$
$$-\Delta H = -9.4 \text{ kg-cal.}$$

From a consideration of these reactions it is apparent that energy is necessary to synthesize fat from carbohydrates, and that amino acids may be formed from carbohydrate with or without expenditure of energy depending upon the source of nitrogen, complexity of the acid, and the availability of oxygen or of hydrogen acceptors. Conversely, the production of carbohydrate from fats would liberate energy, etc. If the proper amino acids, fatty acids, and simple sugars are available, the steps to convert these into protein, esters, and polysaccharides involve little or no energy changes (26, 66).

Experimental data that would help to solve the problem of the energy expenditure for syntheses are sadly lacking. Lundin (42), Meyerhof (47), and others have shown that yeast in the presence of oxygen can convert alcohol and lactic acid into glycogen and an "unhydrolyzable" carbohydrate and that these syntheses apparently take place exothermically. Attempts have been made to calculate the energy required for synthesis from the difference between the heat liberated in fermentations by growing and non-growing cells, but the data are too few to draw definite conclusions. From Rubner's (61) data on the heat of fermentation of sucrose by proliferating and non-proliferating yeast cells, it appears that only 4.1 per cent of the energy liberated in the fermentation is stored in the cells. The data of Brown (8) on fermentation of maltose show, however, that 8.8 per cent less heat is liberated by growing than by non-growing yeast cells. In all of these experiments, the substrate used was equivalent to a mixture of amino acids (peptone, beer-wort). Additional experiments of a similar nature with bacteria, in which the substrate is varied, are necessary before a definite answer can be given to the question of how much energy is required for synthetic purposes. Especially desirable for this purpose are accurate data from experiments in which the bacteria synthesize their

protoplasm from simple organic carbon sources as glucose and an inorganic nitrogen source, e.g., ammonia or nitrate nitrogen.

In the foregoing discussion of the energy required for growth it should be pointed out that two types of energy efficiency have been considered. In the theoretical equations, the free energy available to the organism as shown by the energy balances is the maximum useful work given by the reaction, and the efficiency of its use by the organism will depend on the reversibility of the reaction involved, i.e., the presence or absence of simultaneous, irreversible, frictional processes that lead to dissipation, ordinarily as heat, of part of the free energy liberated. However, the data given are concerned with the efficiency of total processes, i.e., the percentage of the maximum total energy available that can be accounted for by the organism in the synthesis of body matter etc. Due to the irreversibility and hence heat losses attendant on these syntheses, the efficiency indicated by the data is fairly low. Burk (11a) has stressed the importance of this distinction of efficiency in a recent paper dealing with a proof of the validity of the second law in a biological process. He emphasizes the point that in a study of the efficiency of a given reaction from a point of view of the energy relations, it is often desirable, where possible, to separate and to correct for any simultaneous, irrelevant processes whose high degree of irreversibility leads to a low value for the observed efficiency. In every process studied, two types of efficiency are present which he has termed "machine efficiency" and "second law efficiency." "Machine efficiency" is defined as the total work done in the process (chemical or otherwise) divided by the total free energy dissipated by the organism in carrying out all of its life processes. The data in table 3 dealing with the percentage recovery, as bacterial dry matter, of the total energy that disappears from the medium, are examples of "machine efficiency." "Second law efficiency" is defined as the total work done divided by the free energy dissipated solely in the reaction studied. Unfortunately it is not always possible to calculate the second law efficiency because of inability to separate the energy relations of the process being studied from those of other simultaneous processes.

Burk, however, has been able to make a complete energy balance sheet for the autotrophic hydrogen oxidizer, *B. pycnoticus*. He showed that: (*1*) the "machine efficiency" of this organism varies widely with change in experimental conditions and has an average of 28.4 per cent; (*2*) if proper correction is made for extraneous irreversible processes, the efficiency of the coupled reactions involving the oxidation of hydrogen and the reduction of carbon dioxide by this organism is practically perfect (i.e., about 100 per cent) in its reversibility and independent of physiological experimental conditions. In some other processes considered (including autotrophic methane oxidation, heterotrophic nitrate reduction, photosynthesis of carbon dioxide, muscle contraction) it was not possible to secure evidence for 100 per cent efficiency, presumably due to the lack of sufficient or reliable data to make the necessary corrections.

Burk, however, suggests another possibility that would explain the observed lower efficiency in certain cases, depending on what was termed the "biochemical reversibility principle," a principle which stands in relation to the second law of thermodynamics as the second law does to the first law, viz., as conditioning the convertibility of energy from one form to another. The coupled reaction for the heterotrophic reduction of nitrate can be written:

$$H^+ + HNO_3 + H_2O \rightarrow NH_4^+ + 2\ O_2 - \Delta F = -68.0 \text{ kg-cal.}$$
$$X\ O_2 + X\ CH_2O \rightarrow X\ H_2O + X\ CO_2 - \Delta F = 115.0\ X \text{ kg-cal.}$$

With perfect reversibility (100 per cent efficiency) X would equal $\frac{68}{115} = 0.59$. However, the experimental results indicate that X equals 2.0, which is the number of moles oxygen liberated in the energy-requiring process (corresponding to a "second law efficiency" of about 32 per cent). Similarly with the oxidation of hydrogen and reduction of carbon dioxide, the energy relations indicated that X should equal 0.97, which was also that demanded by the stoichiometry of the energy-requiring equation, viz:

$$CO_2 + 0.93\ H_2O \rightarrow \frac{1}{6}\ (C_6H_{.93 \times 12}O_6) + \frac{1.93}{2}\ O_2$$

In this case, as already noted, the observed "second law efficiency" was 100 per cent. These two cases suggest that the maximum "second law efficiency" to be expected in isolated coupled reactions is determined not only by the energy relations of the two reactions but also by the stoichiometry of the reactions. This means that the amount of the energy-yielding reaction that takes place is determined not solely by the free energy demanded by the energy-requiring process, but also by the stoichiometry of this latter process. Quoting Burk, "Expressed in more strictly chemical terms, all the incidental compound produced in the free energy-requiring reaction (in the above particular cases, oxygen) must, by virtue of the mechanism chosen by the organism, be consumed in the free energy-yielding reaction." Although these two reactions are the only ones with sufficient data to test this principle, its implications should be kept in mind as a possible explanation of apparent "wastefulness" of free energy by microörganisms. It is hoped that eventually some of the processes of heterotrophic organisms can be investigated in greater detail by this isolation principle proposed by Burk, and that definite information will replace the present rough approximation that reversibility in heterotrophic organisms is between 0 and 50 per cent.

Energy of motion. At first thought one might expect that much of the energy available to the organisms would be used in their motion. Indeed, Rubner attributed differences in energy of maintenance of growing compared with non-proliferating cells to be due to the difference in motility. Calculations, however, indicate that the energy expended in moving is infinitesimal in comparison with that usually available. Angerer (1) has calculated this by an indirect method; by means of Stokes' law, he estimated the rate at which an organism would fall in a medium. The force responsible for this fall is that of gravity (981 dynes = 1 gram). By a comparison of the rate of fall under gravity and the rate of motion, he estimated the force necessary to move the bacteria. The authors have made a direct calculation by use of a formula in hydrodynamics (37) that gives the force necessary to propel an ellipsoid of the prolate spheroid type, a form that many bacteria approach in their morphology, through a medium of known viscosity.

$$F = 6\pi\mu RV$$

$$R = \frac{8}{3}\left(\frac{abc}{X_0 + \alpha_0 a^2}\right)$$

$$X_0 = abc\int_0^\infty \frac{d\lambda}{\Delta}$$

$$= \frac{2ab^2}{\sqrt{a^2 - b^2}} \ln\left[\frac{a + \sqrt{a^2 - b^2}}{b}\right] \text{ when } b = c$$

$$\alpha_0 = abc\int_0^\infty \frac{d\lambda}{(a^2 + \lambda)\Delta}$$

$$= \frac{2ab^2}{\sqrt{(a^2 - b^2)^3}}\left[\ln\frac{b}{a - \sqrt{a^2 - b^2}} - \frac{\sqrt{a^2 - b^2}}{a}\right] \text{ when } b = c$$

where μ equals viscosity of liquid; V equals velocity of motion; a, b, c equal semi-axes; and R equals effective radius defined in terms of a, b, c, X_0 and α_0; X_0 and α_0 are defined[3] in terms of the definite integrals in which $\Delta = \sqrt{(a^2 + \lambda)(b^2 + \lambda)(c^2 + \lambda)}$.

Table 4 gives a comparison of the data found by the two methods. The authors' calculations were based on the assumption that the bacteria are prolate spheroids with a density equal to 1.055. Although the agreement between the two methods of calculation is not absolute, the results are of the same order of magnitude and indicate that the energy expended for movement by bacteria is only an extremely small portion of that transformed. If the average energy of maintenance is regarded as about 20 kg-cal. per gram of bacterial nitrogen per day, and if the nitrogen content of the wet cells is taken as approximately 2.5 per cent, the energy of maintenance will be about 500 g-cal. per gram of wet bacteria per day. Of this, less than 0.5 g-cal. are used for

[3] It should be noted that α_0 and X_0 are not functions of λ which is merely an integration parameter. Thus it is not necessary to give physical significance to λ. If, however, one writes $\frac{x^2}{a^2 + \lambda} + \frac{y^2}{b^2 + \lambda} + \frac{z^2}{c^2 + \lambda} = 1$, then for given values (x_0, y_0, z_0) of x, y, and z, this equation is a cubic in λ whose three roots are defined as the ellipsoidal coördinates of the point x_0, y_0, z_0.

motion by the sluggish organisms and 1 to 2 g-cal. by the more speedy types. In these calculations it is assumed that the means of locomotion are 100 per cent efficient. This probably is not the case, but even with an efficiency of only 10 per cent the energy

TABLE 4

Energy required for movement by different bacteria

ORGANISM	VELOCITY	FORCE		WORK DONE PER GRAM BACTERIA PER DAY	
		Angerer	Authors	Angerer	Authors
	centimeter per second	*grams* $\times 10^{11}$	*grams* $\times 10^{11}$	*gram-calories*	*gram-calories*
V. cholerae (2.0μ × 0.4 μ)	0.003	8.6	2.06	1.63	0.71
B. typhosus (2.1μ × 0.7μ)	0.0018	5.4*	1.70	0.22*	0.11
B. subtilis (2.1μ × 1.0μ)	0.001	3.0	1.18	0.03	0.02

* This figure was recalculated from Angerer's data, as his calculation contained a numerical error.

TABLE 5

Relationship between oxygen consumption and motility of bacteria

Wohlfeil

ORGANISM	MOTILITY	OXYGEN CONSUMPTION PER HOUR PER 10^9 ORGANISMS
		cc. $\times 10^7$
Experiment 1:		
Bac. dysenteriae (Shiga)	−	6.80
Bac. enteritidis (Gärtner)	±	6.74
B. coli commune	±	8.41
Bac. typhosus	+	8.44
Bac. paratyphosus B	+++	9.10
B. faecalis alcaligenes	++++	11.54
Experiment 2—*B. coli commune:*		
Strain 1		5.40
Strain 2		4.06
Strain 3		3.87
Strain 4		3.35

expended in motion is an inconsiderable share of the total available.

Of interest in this connection are data published recently by Wohlfeil (73), who measured the oxygen consumption of bacteria

which are closely related but which exhibit varying degrees of motility. His data are summarized in table 5.

From the data of the second experiment in which four strains of *B. coli* were used, Wohlfeil decided that the oxygen consumption is fairly constant among members of the same species, therefore the difference noted among the organisms of experiment 1 constituted real and not apparent differences. He assumed that the difference noted in the oxygen uptake by *Bact. dysenteriae* and *Bac. typhosus* is due to the motility of the latter with a consequent higher energy requirement. He converted the difference into gram-calories by use of the relationship 1 cc. O_2 = 4.67 g-cal., and thus calculated the energy required for motion by *Bac. typhosus* to be 2.15×10^{-19} g-cal. per second per bacterium. He noted that this result agrees remarkably well with the figure calculated by Angerer for the energy requirements of this organism for motion, viz., 2.80×10^{-19} g-cal. per second. Unfortunately there is an error in the calculation of Angerer for this organism. The correct value for the energy requirements for motion by *Bac. typhosus* calculated according to Angerer's method is 23.0×10^{-19} g-cal. per second per bacterium. Calculated according to the formula given on page 447 this is reduced to 7.2×10^{-19} g-cal. per second per bacterium. However, the difference between the experimental value of Wohlfeil and these calculated values may be due to the fact that not all of the organisms are in motion, as is assumed in the calculations. Wohlfeil mentions that random fields of *Bac. typhosus* showed only one out of five or even one out of ten of the organisms in motion.

A more severe criticism of Wohlfeil's data is that of the statistician. The mean of the four strains of *B. coli* is 4.7×10^{-7} cc. oxygen per hour per 10^9 bacteria. The standard deviation of the individual values is 0.754×10^{-7} or about 18 per cent of the mean. While it would be highly desirable to have more than four samples for the calculation of these statistics, the data available suggest that differences from the mean of 36 per cent or less (twice the standard deviation) may well arise from chance. *Bac. typhosus* had an oxygen consumption only 24 per cent higher than that observed for *Bac. dysenteriae*. In view of the variations noted

among the strains of *B. coli*, this difference cannot be considered significant as it stands. The variation among the strains of *B. coli* might have arisen from differences in motion by these strains since Wohlfeil reports their motility as ±, i.e., they are only partly motile. In spite of these objections, the data of Wohlfeil are not without value; it appears that there is a high positive correlation between motility and oxygen consumption. Further work, in which a number of strains of each species are used in order to control the individual differences, might yield extremely interesting and more conclusive results.

Energy for increase of surface. When the question of the energy expended in increasing the surface of bacteria during growth is considered, the lack of data on the interfacial tension between the cells and the medium requires the use of indirect methods that may or may not yield valid conclusions. A bacterium 2μ in length and 1μ in diameter, considered as a cylinder, would have a surface of 7.85×10^{-8} sq. cm. and a volume of 1.57×10^{-12} cc. Thus it would require 6.2×10^{11} wet bacteria to weigh 1 gram (density = 1.055). This 1 gram of bacteria would have a surface equal to 4.88×10^{4} sq. cm. Since 1 calorie = 4.182×10^{7} ergs, the work of adhesion between the cell and the medium (disregarding the small amount of surface present in the inoculum) would have to be $\frac{4.182 \times 10^{7}}{4.88 \times 10^{4}} = 857$ ergs per sq. cm. in order to use 1 g-cal. equivalent of energy in the production of the surface of 1 gram of bacteria of the above dimensions. In like manner, the work of adhesion necessary to produce the surface represented by 1 gram of wet bacteria of the dimensions 1.0μ by 0.5μ would be 441 ergs per sq. cm. if 1 g-cal. equivalent of energy were used in the process. These values are much higher than those found by Harkins (25) for the work of adhesion between organic liquids and water. Tangl (65) reports that the total energy expended in the production of 1 gram of bacteria is 2000 to 4000 g-cal., consequently on the basis of the foregoing data, it appears that the work done in increasing the surface must be small in comparison with this total. It should not be overlooked that the work of adhesion between the cell membrane-medium interface might be markedly

different from the organic liquid-water interfaces studied by Harkins. However, there is some indirect evidence in support of the view that the interfacial tension between the cell and the medium is rather low. Davis (16) found that the effect of various cations on the size of oil drops (oleic acid) in water paralleled their effect on the growth of *B. coli*. He suggests that the surface tension of bacteria is essentially that of a water-oil interface. If this is true, then it can be concluded that only a fraction of a g-cal. is necessary to increase the (external) surface of 1 gram of bacteria (in contrast to the several thousand calories available). Support of this low value for the interfacial tension between bacterium and water is offered by the work of Mudd and Mudd (49). These workers studied the behavior of bacteria in water-oil interfaces. Their data suggest that most bacteria act as a semi-solid gel with polar groups oriented in the surface so that the interfacial tension between the cell and water is rather low. Acid-fast organisms, however, have a smaller interfacial tension toward the oil phase than toward water. The calculations of the surface formed in the production of 1 gram of bacteria involved the assumption that this surface was smooth. If the cell membrane is sponge-like in character, as is a silica gel, then the surface area would be greatly increased. In spite of these assumptions, it seems likely that the work done in the production of the external surface of bacteria uses only a small part of the total energy available.

Other expenditures of energy. From the foregoing discussion, it must be concluded that the available information concerning the manner of expenditure of energy by the cell is of a rather negative character. It appears that only a small part of the energy that is transformed can be accounted for; the remainder of the energy finally appears as heat, but it is not known whether this heat arises directly from the chemical energy liberated or is the result of work done by it. Warburg (71) has determined the quotient $\frac{\text{work done}}{\text{maximal work available}}$ for a number of cells, and has found that except in the case of the active muscle this quotient was extremely small. He attributes this to the fact that work

is being done that is not apparent. Examples of this type of work would be: retention of phase boundaries, creation of internal surfaces, prevention of diffusion, and osmotic work done in transportation of substances from a region of low concentration to a region of higher. Nathanson (50) has suggested that bioelectric potentials originate in cells due to differences in concentration of ions on either side of a membrane or through selective adsorption. This potential is then capable of decomposing water, and by immediate depolarization a supply of nascent oxygen is maintained for cellular oxidation. This electric force is also the means of supplying the energy for secretion and adsorption. If this hypothesis is correct, then part of the energy unaccounted for is used in the production and maintenance of unequal concentrations on either side of the membrane in order to produce the necessary potential. In this connection it must be remembered that the change of free energy of all processes at the equilibrium point is zero. All the reactions taking place in the cell are moving toward equilibrium, hence work must be done continually to keep these equilibrium points from being reached so that the metabolic processes can continue.

Giaja (21) has reported that the "fundamental biologic energy" of yeast—that is, the energy necessary to maintain the cells when no external work is done—is much smaller than that liberated by yeast in the presence of sugar. He regards the energy liberated when non-proliferating cells are placed in sugar solutions as due to uncontrolled activity of the enzymes present in the cell. A purpose for this apparent excess liberation of energy was suggested by Brown (8). He found that yeast required only 2.2 hours to ferment its own weight of maltose, and calculated that the metabolism of yeast must be about seventy times that of man. If the heat liberated by the yeast in its metabolism were not dissipated into the surrounding medium, the temperature of the cell would be raised 75.5°C. per hour at 30°C. Brown attributed this large heat liberation to the fact that in the natural habitat of the organism the heat losses are so great that such liberation of heat is required to keep the cell at the optimum temperature. When the yeast is placed in the artificial environ-

ment of the laboratory medium, this excess energy is no longer needed, but is liberated because of the lack of control of the cell over its enzymatic activities.

These conclusions are based on the assumption that the cell liberates its heat under strictly adiabatic conditions. However, unless the organism is fairly well insulated, it appears that even the high rate of heat production noted by Brown would be insufficient to maintain its temperature much above that of the surrounding soil, fruit, or other natural substrate. An approximation of the rate of heat production necessary to keep a colony of yeast cells at a temperature higher than that of its environment can be made by the following formula (30). In the application the colony of yeast is considered as a sphere surrounded by a spherical coating of soil.

$$E = \frac{4 \pi k (T_1 - T_2) r_1 r_2}{r_1 - r_2}$$

E = rate of heat production in gram-calories per second necessary to produce a temperature difference between surfaces of the two spheres,
k_1 = heat conductivity of soil (dry soil = 0.00033),
r_1 = radius in centimeters of outer sphere (soil + colony),
r_2 = radius in centimeters of inner sphere (colony),
$T_1 - T_2$ = difference in temperature of the surfaces of the inner and outer spheres.

Table 6 gives a summary of calculations made for colonies of various radii surrounded by a layer of soil of different thicknesses.

These figures show that only large colonies with thick layers of insulation are able to maintain a temperature above that of the environment. Small colonies, because of the large surface per unit volume, are unable to retain the heat liberated in the metabolism of the cells. The formula shows that for a given insulating substance of heat conductivity k, the rate of heat production necessary to keep the temperature of the surface of the inner sphere 1°C. higher than that of the outer sphere approaches a minimum equal to $4\pi kr_2$ as r_1 is increased. The calculations show that for a colony 1 mm. in radius the potential difference in

temperature increases from 0.04°C. to 0.19°C. as the layer of soil is increased from 0.25 mm. to 99.0 mm. Also the temperature difference changes very little with further increase in the thickness of the soil layer. It follows that within certain limits (from two to ten times the radius of the inner sphere) it makes little difference as to what is assumed for the thickness of the soil layer. In the above calculations the figure for the heat conductivity of dry soil is used; in a moist soil the conductivity would be higher

TABLE 6

Potential temperature differences between environment and yeast colonies of varying size

RADIUS OF COLONY (r_2)	RADIUS OF COLONY AND SOIL (r_1)	RATE OF HEAT PRODUCTION		TEMPERATURE DIFFERENCE
		Required for difference of 1°C.	Available*	
millimeters	*millimeters*	*gram-calories per second* $\times 10^4$	*gram-calories per second* $\times 10^4$	*°C.*
1	1.25	20.75	0.786	0.04
1	2.0	8.30	0.786	0.10
1	10.0	5.20	0.786	0.15
1	100.0	4.20	0.786	0.19
5	10.0	41.50	98.20	2.36
5	100.0	21.90	98.20	4.48
10	20.0	83.0	786.0	9.46
10	100.0	43.7	786.0	18.00

* Calculated on the assumption that yeast ferments its own weight of maltose in 2.2 hours.

and the potential rise in temperature of the colony correspondingly smaller. In a well-insulated environment or with large masses of cells the rate of heat production by microörganisms is sufficiently high to cause a rise in temperature in the immediate vicinity, as is noted in the biological heating of manure. However, it appears unlikely that microörganisms in general in their natural habitats produce heat at a rate sufficient to maintain an optimum temperature above that of their surroundings.

II. THE APPLICATION OF ENERGETICS TO FERMENTATIVE PROCESSES

Alcoholic fermentation

The energetics of this fermentation have been more completely investigated than any other because of its industrial importance.

Rubner (58, 59) studied the total heat produced, the heat produced by the action of zymase and invertase and determined the influence of alcohol, nitrogen nutrition, and other factors on the energy changes. He found that the heat liberated by zymase was only a small part of the total liberated, and used this as an argument for the theory that most of the fermentation resulted from cells *per se*. A carbon balance indicated that 1 gram of sucrose should liberate about 159.4 g-cal.,[4] whereas that actually measured in the calorimeter was 149.9 g-cal. per gram of sucrose. The calculated value for glucose was 147.6 g-cal.[4] per gram and the measured value was 133.3 g-cal. per gram. The concentration of sugar and of yeast did not affect these values but only the

TABLE 7

Heat production by different species

Rubner

SPECIES	HEAT PRODUCTION PER DAY PER KILO WEIGHT	HEAT PRODUCTION PER DAY PER SQUARE METER OF BODY SURFACE
	kilogram-calories	*kilogram-calories*
Man	30.0	1042
Dog	45.9	1039
Mouse	210.0	1039
Yeast	1163 (30°C.)	0.84–1.2
Yeast	1173 (38°C.)	

rate of heat production; the presence of alcohol resulted in a decrease of the total heat production. Table 7 gives a comparison of the heat produced by different species, although Rubner pointed out that little agreement should be expected between species with different physiological and anatomical characteristics.

Fulmer and Leifson (19) have made a noteworthy contribution to the study of the free energy changes occurring in the formation of alcohol from sucrose. These authors also outlined in detail the methods suitable for calculation of the change in $-\Delta F$ with changes in concentrations of the reactants and resultants and

[4] Calculated from more recent thermal data.

stressed the importance of considering these energy changes when dealing with biological transformations.

From the equation

$$C_{12}H_{22}O_{11}\ (s) + H_2O\ (l) \rightarrow 4\ CO_2\ (g) + 4\ C_2H_5OH\ (l)$$
$$-\Delta F^\circ = 116.6 \text{ kg-cal.}$$

they calculated the change in $-\Delta F$ when 0.3 mole of sucrose is fermented for various initial concentrations of sucrose and ethyl alcohol and for a constant pressure of 0.0003 atm. of carbon dioxide (air). The data in table 8 are taken from their paper.

These data show that the maximum difference between the free energy decrease for concentrations of sucrose, ethyl alcohol, and

TABLE 8

Average values of $(\Delta F - \Delta F^\circ)$* *per mole of sucrose fermented when* $-\Delta M = 0.3$ *mole*

Fulmer and Leifson

INITIAL CONCENTRATION OF SUCROSE	INITIAL CONCENTRATION OF ETHYL ALCOHOL		
	$2.1 \times 10^{-4} M$	$2.1 \times 10^{-2} M$	$2.1 \times 10^{-1} M$
M†	*kilogram-calories*	*kilogram-calories*	*kilogram-calories*
1.00	−27.42	−24.65	−23.02
0.70	−26.52	−23.75	−22.61
0.50	−24.95	−23.36	−22.08

* $-\Delta F$ = decrease in free energy under conditions indicated in table.
$-\Delta F^\circ$ = decrease in free energy in standard states = 116.6 kilogram-calories.

† M = molality.

carbon dioxide likely to be present in a fermentation and the $-\Delta F^\circ$ (standard states) is about 27 kg-cal., or an increase of 23 per cent. The authors conclude, "Considering . . . . the fact that the equation assumed for the fermentation does not represent accurately the products formed by biological action, the corrections from the standard state in this case are not of relatively great importance. However, it is worth while, in each instance, in the use of free energy changes of biological processes to analyze the situation along the lines presented above."

Lactic acid fermentation

Rubner (60) studied the heat produced by this fermentation both in pure culture and in the spontaneous souring of milk.

Experiments made on the souring of milk showed that only 25 to 45 per cent of the observed heat production could be accounted for by the theoretical equation for the decomposition of lactose into lactic acid. Using sterilized milk inoculated with an "organism producing dextro-lactic acid" he found only 23 per cent of the observed heat could be accounted for by the acid produced. He suggests that other vital processes yielding heat caused these discrepancies, and believes that for this reason heat production is a better method for comparing the metabolism of these organisms than is lactic acid production.

Acetone-butyl alcohol fermentation

A study of the mechanism of this fermentation made in this laboratory (31) has indicated certain theoretical equations for the break-down of carbohydrates into the solvents and acids formed in the fermentation. From data on the fermentation of glucose it was found that the concentrations of acetic and butyric acids varied so little from the tenth to the thirty-second hour that the concentrations may be treated as constant during this period. Also 40 per cent of the glucose disappeared during this time, so that the free energy changes of this period may be taken as fairly representative of the entire fermentation. The free energy equations for the production of the acids showed that the change in the glucose concentration did not materially affect the total decrease in free energy, hence the average value of the glucose was used to calculate the free energy changes involved in the formation of solvents. In the following equations each fermentation product is referred to an individual molecule of glucose for purposes of calculation. In the actual fermentation these products arise undoubtedly from a common intermediate compound rather than each from a discrete glucose molecule.

$$\underset{\text{Acetic acid}}{}$$

$$C_6H_{12}O_6 \begin{Bmatrix} 0.115 \\ 0.060 \end{Bmatrix} M \Big\} + 2\ H_2O\ (l) \rightarrow 2\ CH_3COOH\ (0.035M) + 2\ CO_2\ (0.65\ \text{atm.}) + 4\ H_2\ (0.35\ \text{atm.})$$

$$-\Delta F = \begin{Bmatrix} 54.3 \\ 53.9 \end{Bmatrix} \text{kg-cal.}, \quad -\Delta H = -17.5\ \text{kg-cal.}$$

Butyric acid

$$C_6H_{12}O_6 \begin{Bmatrix} 0.115 \\ 0.060 \end{Bmatrix} M \rightarrow C_3H_7COOH\ (0.015M) + 2\ CO_2\ (0.65\ \text{atm.}) + 2\ H_2\ (0.35\ \text{atm.})$$

$$-\Delta F = \begin{Bmatrix} 69.0 \\ 68.6 \end{Bmatrix} \text{kg-cal.}, \quad -\Delta H = 17.2\ \text{kg-cal.}$$

Acetone

$$C_6H_{12}O_6\ (0.087M) + H_2O\ (l) \rightarrow CH_3COCH_3 \begin{Bmatrix} 0.003 \\ 0.020 \end{Bmatrix} M + 3\ CO_2\ (0.65\ \text{atm.}) + 4\ H_2\ (0.35\ \text{atm.})$$

$$-\Delta F = \begin{Bmatrix} 49.5 \\ 48.4 \end{Bmatrix} \text{kg-cal.}, \quad -\Delta H = -26.4\ \text{kg-cal.}$$

Butyl alcohol

$$C_6H_{12}O_6\ (0.087\ M) \rightarrow C_4H_9OH \begin{Bmatrix} 0.004 \\ 0.034 \end{Bmatrix} M + 2\ CO_2\ (0.65\ \text{atm.}) + H_2O\ (l)$$

$$-\Delta F = \begin{Bmatrix} 70.7 \\ 69.4 \end{Bmatrix} \text{kg-cal.}, \quad -\Delta H = 35.0\ \text{kg-cal.}$$

Ethyl alcohol

$$C_6H_{12}O_6\ (0.087\ M) \rightarrow 2\ C_2H_5OH \begin{Bmatrix} 0.003 \\ 0.017 \end{Bmatrix} M + 2\ CO_2\ (0.65\ \text{atm.})$$

$$-\Delta F = \begin{Bmatrix} 58.7 \\ 57.6 \end{Bmatrix} \text{kg-cal.}, \quad -\Delta H = 18.1\ \text{kg-cal.}$$

From these data it was calculated that from the tenth to the thirty-second hour 0.055 moles of glucose were decomposed with a free energy decrease of 3.34 kg-cal., or $-\Delta F = 60.8$ kg-cal. per mole of glucose fermented. Since the standard free energy of the complete oxidation of glucose is -685.8 kg-cal. per mole, it is seen that the anaerobic decomposition by this organism liberates less than one-tenth of the "potential" free energy. The production of acetic acid and acetone from glucose are examples of reactions in which $-\Delta F$ and $-\Delta H$ have opposite signs.

Of interest is the consideration of two of the proposed steps in the mechanism. Acetone is thought to be formed from acetic acid. As can be seen from the following equation, this reaction has a positive ΔF, hence energy from another reaction would be necessary to cause it to take place. This necessary energy could be obtained from that liberated in the formation of the acetic acid from glucose.

$$2\ CH_3COOH\ (0.035\ M) \rightarrow CH_3COCH_3 \begin{Bmatrix} 0.003 \\ 0.020 \end{Bmatrix} M + CO_2\ (0.65\ \text{atm.}) + H_2O\ (l)$$

$$-\Delta F = \begin{Bmatrix} -4.7 \\ -5.9 \end{Bmatrix} \text{kg-cal.}, \quad -\Delta H = -9.0\ \text{kg-cal.}$$

The reduction of butyric acid to butyl alcohol may require nascent hydrogen. Using the figure given by Lewis for the standard free energy of atomic hydrogen, it appears that the production of butyl alcohol also involves a coupled reaction.

$$C_6H_{12}O_6 \rightarrow C_3H_7COOH + 2\ CO_2 + 4\ (H)$$
$$-\Delta F^\circ = -88.9 \text{ kg-cal.}$$
$$C_3H_7COOH + 4\ (H) \rightarrow C_4H_9OH + H_2O$$
$$-\Delta F^\circ = 169.0 \text{ kg-cal.}$$

The first reaction, which requires large quantities of energy to cause it to take place, could obtain this energy from that liberated in the second reaction. In all of these reactions, it is assumed that the concentrations of the reactants and resultants are identical with those of the circumambient liquid. This condition in all probability does not obtain, since the cell apparently alters the concentrations at its active surfaces by performing osmotic work. If the concentrations are changed, the free energy liberated likewise will be changed. However, until more definite information is available, calculations of this nature must serve as first approximations of the free energy change.

Other bacterial fermentations

As pointed out in Part I, if the products of a fermentation are accurately determined, it is possible by an application of thermodynamical data to calculate the heat of reaction and free energy liberated as a result of the fermentation. The energetics of a number of fermentations given in the literature have been calculated by this method. In choosing the data two criteria have been used: (*1*) the carbon of the products must equal at least 95 per cent of the carbon of the carbohydrate decomposed; (*2*) the oxidation reactions must balance the reduction reactions at least to within 5 per cent. If the original data fulfilled these conditions they were then adjusted so that the oxidations and reductions were exactly equal: this was most conveniently done by correcting the carbon dioxide or hydrogen figure. After this adjustment, a carbon balance was made by correcting the figure for the carbohydrate fermented so that its carbon was equal to that present in the products. This corrected figure was then com-

pared with the original "carbohydrate fermented" and, if it was within 5 per cent of the latter, the data were judged to be sufficiently accurate for our purpose. These adjustments are necessary since small differences in carbon or oxidation-reduction balances will cause a large difference in the calculated value of the energy liberated. Finally the water that took part in the reaction was calculated by making an oxygen or hydrogen balance, and the entire data were put on the basis of 1000 millimoles (1 mole) of carbohydrate fermented in order to facilitate subsequent calculations.

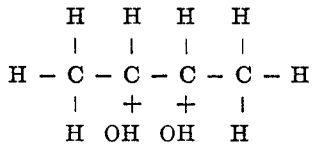
In calculating the standard free energy of the reaction, it was necessary to estimate the free energy of formation of propionic acid, 2,3-butylene glycol, and arabinose, since these data have not been determined. The value for propionic acid was taken to be the average of those for butyric and acetic acids, since Parks and Kelley (54) have shown that the introduction of a CH_2 group in a homologous series causes little change in the value of the free energy of formation. The values for the other compounds were calculated by the "atomic entropy" method of Parks and Kelley as described by Burk (12). Dr. Kelley kindly furnished us with constants based on specific heat measurements of a number of organic compounds down to a temperature of 15°K. These constants are not the same as those given by Burk since the latter are based on earlier, less accurate measurements. It was also necessary to calculate the heat of combustion of 2,3-butylene glycol. This was done by the method of Kharasch (32).

As an example of the use of these methods the calculations for 2,3-butylene glycol are given in detail.

(*1*). *The heat of combustion of an organic compound.* Kharasch has conceived the oxidation of organic compounds as a process in which energy is released when electrons are displaced from orbits occupied in the methane type of molecules to orbits characteristic of the carbon dioxide type. This change liberates energy equal to 26.05 kg-cal. per electron per mole. In the case of molecules that have substituted groups, the orbits are already partially displaced; hence it is necessary to apply a correction

factor to the general formula, $Q = 26.05 \times N$, where Q = heat of combustion in kg-cal., and N = number of electrons displaced per mole. These correction factors have been worked out by Kharasch and his collaborators, who have shown that the calculated values of the heats of combustion are accurate to within 1 per cent of the experimentally determined values.

From the structural formula of 2,3-butylene glycol the number of electrons displaced is determined (33).



Each hydrogen is positive, hence would be connected with a minus sign to carbon. Carbon to carbon is considered as one negative bond. The oxygens are negative, therefore the connection to carbon is positive. The total number of electrons displaced is the sum of the negative bonds (11) times 2. Kharasch gives the following formula for a polyhydroxy alcohol,

$$Q = 26.05 \times N + 13\ j + 6.5\ k + 3.5\ l,$$

where j equals the number of primary alcohol groups, k equals the number of secondary alcohol groups, and l equals the tertiary alcohol groups. In the case of 2,3-butylene glycol, N equals 22, j and l equal 0, and k equals 2. Therefore,

$$\begin{aligned} Q &= 26.05 \times 22 + 2 \times 6.5 \\ &= 586.1 \text{ kg-cal.} \end{aligned}$$

The heat of formation is calculated from the formula,

$$-\Delta H = -Q + 94.38\ C + 68.38\ H,$$

where Q equals the heat of combustion of the compound, C equals the number of carbon atoms in the compound, and H equals the number of hydrogen moles in the compound. Therefore,

$$\begin{aligned} -\Delta H &= -586.1 + 94.38 \times (4) + 68.38 \times (5) \\ &= 133.3 \text{ kg-cal.} \end{aligned}$$

(*2*). *The free energy of formation of an organic compound.* In order to calculate the free energy of formation it is necessary to know the entropy at 25°C. This can be calculated from the structural formula in a manner similar to the calculation of molecular refraction, volume, etc. The necessary constants were furnished to the authors by Dr. Kelley, who states "For the liquid, aliphatic, saturated alcohols (including polyalcohols), acids and ketones, the following values for 298°K. satisfactorily reproduce the entropies of all the compounds on which we have data:

$$H\text{—}\ (+10.6);\ \text{—}\overset{|}{\underset{|}{C}}\text{—}\ (-13.6);\ \text{—}O\text{—}\ (+2.0);\ =O\ (+21.2)$$

For each secondary hydroxyl group three units should be subtracted from the value calculated from these constants (based on one result). These constants have little theoretical meaning, but are obtained empirically by solving simultaneous composition equations. Consequently, I believe you can strengthen your case by obtaining the values you desire by comparison, when possible, with similar compounds which have been studied."[5]

This last statement can be illustrated by the calculation used for solid arabinose. Glucose has an entropy value of 50.5 units at 25°C. The data on solid ethylene glycol and glycerol when compared with the figures for erythritol, mannitol, and dulcitol indicate that each CHOH group (in the solid state) is equivalent to 6 or 7 units. Hence the entropy of arabinose was calculated by subtracting 6.5 units from that of glucose; from the value obtained (44.0 E.U.) the free energy of formation of solid arabinose can be calculated. The atomic entropy of 2,3-butylene glycol was calculated:

$4 \times (-13.6)$	=	-54.4	for 4 carbon atoms
$10 \times (\ 10.6)$	=	106.0	for 10 hydrogen atoms
$2 \times (\ 2.0)$	=	4.0	for 2 oxygen atoms
$2 \times (-3.0)$	=	-6.0	for 2 CHOH groups
	=	49.6 E.U.	

[5] Private communication to the authors.

This value may be somewhat in error since the correction for the secondary hydroxyl group is based on only one case, viz., the difference between propyl and isopropyl alcohol. From this value for the entropy of 2,3-butylene glycol, the decrease of entropy when this compound is formed from the elements can be calculated if the entropies of the elements are available. Parks, Kelley, and Huffman (55) give the following values for the entropies of the necessary elements: C equals 1.3 E.U. per gram-atom; H equals 14.8;[6] and O equals 24.5. For $CH_3(CHOH)_2CH_3$ the calculation is:

$4 \times 1.3 =$	5.2
$10 \times 14.8 =$	148.0
$2 \times 24.5 =$	49.0
Sum of entropies of elements =	202.2
Entropy of 2,3-butylene glycol =	49.6
$-\Delta S_{298}$ of formation =	152.6 E.U.

From the relationship $\Delta F = \Delta H - T\Delta S$ the free energy of formation is readily obtained.

$$\Delta F^\circ_{298} = -133.3 + 298 \times 0.1526$$
$$= -87.8 \text{ kg-cal.}$$

These calculated values are probably correct to within 2 to 3 per cent. The data for the heats of combustion for the other compounds are those given in the International Critical Tables and by Kharasch; the $-\Delta F^\circ$ values are those of Parks, Kelley, and Hoffman (55).

[6] The value for hydrogen that is now accepted is that of Giauque (J. Am. Chem. Soc. **52**, 4816 (1930)), who reports that this value should be 31.23 units per mole instead of 2×14.8 as formerly used. However, since the free energy values of all the other compounds considered in this paper are based on the old value for hydrogen, it was decided to use 14.8 per atom in the calculation of the free energy of formation of 2,3-butylene glycol. In any case the effect of changing the entropy of hydrogen will affect only those reactions in which elemental hydrogen enters as a reactant or resultant. Moreover, the revision necessary for the free energy values of organic compounds, in light of the new value for the entropy of hydrogen, will be of too small an order to affect calculations presented in this paper. Thus, in the case of glucose, the change is less than 1 per cent and would be much the same for similar organic compounds.

The results are given in tables 9, 10, 11, 12, and 13. Table 9 shows the details of the method used in the calculations of energy balances of two fermentations. In the remaining tables only the summaries are given. *B. lactis aerogenes* (table 10) liberates very little heat—3.1 to 5.4 kg-cal. in the fermentation of glycerol—but the maximum free energy available for use is higher. The

TABLE 9

Detailed calculations of energy balances of two fermentations

SUBSTRATE AND ORGANISM	*B. coli* ON GLYCEROL			*B. coli* ON GLUCOSE		
FERMENTATION BALANCE		$-\Delta H \times$ mole fraction	$-\Delta F^\circ \times$ mole fraction		$-\Delta H \times$ mole fraction	$-\Delta F^\circ \times$ mole fraction
	millimoles	*kilogram-calories*	*kilogram-calories*	*millimoles*	*kilogram-calories*	*kilogram-calories*
Compound fermented	1000	−159.7	−115.7	1000	−302.5	−219.0
Products formed:						
Formic acid	125	12.50	10.65	163	16.30	13.89
Acetic acid	90	10.49	8.59	480	55.92	45.79
Lactic acid	128	20.75	16.15	916	148.65	115.60
Succinic acid	31	6.99	5.60	87	19.59	15.70
Ethyl alcohol	688	45.31	29.72	536	35.32	23.16
2,3-Butylene glycol	99	13.20	8.70	—	—	—
Hydrogen	313	—	—	596	—	—
Carbon dioxide	415	39.17	39.12	709	66.91	66.83
Water	346	23.66	19.57	−336	−22.98	−19.00
$\Sigma -\Delta H$ products × mole fraction of each		172.1			319.7	
$-\Delta H$ of fermentation per mole substrate		12.4			17.2	
$\Sigma -\Delta F^\circ$ products × mole fraction of each			138.1			262.0
$-\Delta F^\circ$ of fermentation of 1 mole of substrate			22.4			43.0

data for *B. coli* in the same table indicate that, while the heats of reaction in the decomposition of glycerol and of glucose are much the same, the free energy liberated is much greater in the fermentation of glucose. This is due to qualitative and quantitative differences in the products formed. No definite conclusions can be reached as to the constancy of this difference since it may be only a question of variation in strains. However, fermentations

of glycerol by two strains of *B. coli*, one reported by Harden and Norris in 1912, the other by Braack in 1929, give similar results for the energy exchange.

Table 11 presents a study of the energetics of different strains of the propionic acid bacteria on glucose. The data for strains 4,

TABLE 10

Energy balance of coli-aerogenes bacteria

ORGANISM	*B. lactis aerogenes*			*B. coli*	
REFERENCE	(7)	(7)	(7)	(24)*	(22)
SUBSTRATE	GLYCEROL	GLYCEROL	GLYCEROL	GLYCEROL	GLUCOSE
	millimoles	*millimoles*	*millimoles*	*millimoles*	*millimoles*
Total fermented	1000	1000	1000	1000	1000
Products formed:					
Formic acid	130	146	145	125	163
Acetic acid	75	57	31	90	480
Lactic acid	32	10	168	128	916
Succinic acid	65	54	92	31	87
Ethyl alcohol	862	886	756	688	536
Hydrogen	751	872	543	313	596
Carbon dioxide	640	722	409	415	709
Water	92	18	202	346	336†
	kilogram-calories	*kilogram-calories*	*kilogram-calories*	*kilogram-calories*	*kilogram-calories*
$-\Delta H$ of substrate fermented	+159.7	+159.7	+159.7	+159.7	+302.5
$\Sigma -\Delta H$ products × mole fraction of each	165.1	162.8	168.3	172.1	319.7
$-\Delta H$ of fermentation per mole substrate	5.4	3.1	8.6	12.4	17.2
$-\Delta F°$ of substrate fermented	115.71	115.71	115.71	115.71	219.0
$\Sigma -\Delta F°$ products × mole fraction of each	136.9	136.2	135.8	138.1	262.0
$-\Delta F°$ of fermentation per mole substrate	21.2	20.5	20.1	22.4	43.0

* 99 millimoles of 2,3-butylene glycol formed in this fermentation.

† This water reacts with the glucose to form the products.

14, and 12 are taken from the paper by Foote, Fred, and Peterson (18), while those for the other two come from the work of Van Neil (67). In spite of differences in the relative distribution of the acids formed by the various strains, the energy liberated is

practically the same in all cases. The values of $-\Delta H$ and $-\Delta F°$ for this fermentation are much higher than those of any of the others studied.

The data given in table 12 were collected in an effort to ascertain the differences in the energy liberated by the metabolism of the same organism on a number of carbohydrates. The species

TABLE 11

Energy balance of the fermentation of glucose by the propionic acid formers

PROPIONIC ACID FORMERS	STRAIN 4	STRAIN 14	STRAIN 12*	STRAIN 6	STRAIN 22
REFERENCE	(18)	(18)	(18)	(67)	(67)
	millimoles	*millimoles*	*millimoles*	*millimoles*	*millimoles*
Glucose fermented	1000	1000	1000	1000	1000
Products formed:					
Acetic acid	676	717	645	539	651
Propionic acid	1015	1170	1293	1295	1256
Succinic acid	310	134	38	112	87
Carbon dioxide	353	518	628	591	584
Water	672	654	665	702	670
	kilogram-calories	*kilogram-calories*	*kilogram-calories*	*kilogram-calories*	*kilogram-calories*
$-\Delta H$ of glucose fermented	302.5	302.5	302.5	302.5	302.5
$\Sigma -\Delta H$ products $\times$ mole fraction of each	350.8	349.1	347.9	348.6	348.5
$-\Delta H$ of fermentation per mole glucose	48.3	46.6	45.4	46.1	46.0
$-\Delta F°$ of glucose fermented	219.0	219.0	219.0	219.0	219.0
$\Sigma -\Delta F°$ products $\times$ mole fraction of each	288.5	290.0	290.6	290.4	290.4
$-\Delta F°$ of fermentation per mole glucose	69.5	71.0	71.6	71.4	71.4

* 17 moles lactic acid formed in this fermentation.

chosen was *Lactobacillus pentoaceticus*—the so-called mannitol former. It is to be noticed that the energy liberated in the fermentation of glucose is practically the same as that set free from galactose, but the fermentation of fructose, in which mannitol is formed by reduction of the sugar, results in a large decrease in the energy liberated. The fermentation of maltose gives an energy yield very similar to that of glucose. The energy liberated in the

sucrose fermentation is between that of glucose and fructose, as is to be expected.

Table 13 gives the results of a similar study, using the industrial acetone-butyl alcohol organism, *Cl. acetobutylicum*. These data, like those in table 12, show that as the source of carbon is varied,

TABLE 12

Energy balance of the fermentation of sugars by the mannitol formers

REFERENCE	(64)	(64)	(20)	(20)	(68)
CARBOHYDRATE	GLUCOSE	GALACTOSE	FRUCTOSE	MALTOSE*	SUCROSE
	millimoles	*millimoles*	*millimoles*	*millimoles*	*millimoles*
Total fermented	1000	1000	1000	1000	1000
Products formed:					
Acetic acid	99	49	401	314	187
Lactic acid	916	873	309	642	689
Succinic acid	—	—	—	9	15
Ethyl alcohol	1018	1095	—	934	670
Mannitol	—	—	657	—	230
Carbon dioxide	1018	1095	328	1017	778
Water	—	—	−328†	−83†	−106†
	kilogram-calories	*kilogram-calories*	*kilogram-calories*	*kilogram-calories*	*kilogram-calories*
$-\Delta H$ of carbohydrate fermented	302.5	302.5	302.5	302.5	302.5
$\Sigma -\Delta H$ products × mole fraction of each	323.4	323.0	314.4	322.6	320.6
$-\Delta H$ of fermentation per mole carbohydrate	30.9	20.5	11.9	20.1	18.1
$-\Delta F°$ of carbohydrate fermented	219.0	219.0	219.0	219.0	219.0
$\Sigma -\Delta F°$ products × mole fraction of each	265.2	265.4	238.2	264.8	255.9
$-\Delta F°$ of fermentation per mole carbohydrate	46.2	46.4	19.2	45.8	36.9

* 175 moles glycerol formed in this fermentation.

† This water reacts with the sugar to form the products. The maltose was calculated as glucose; the sucrose as 50 millimoles of glucose + 50 millimoles of fructose.

with resulting changes in the products, the energy liberated also shows variations.

It is apparent from the data just discussed that for different fermentations $-\Delta H$ per mole sugar fermented varies from about 5

kg-cal. or less, to as high as 50 kg-cal., while $-\Delta F°$ ranges from 20 to 70 kg-cal. It would appear that either the needs of organisms for energy are much different or that an excess is produced in a large number of cases. No definite conclusion can be drawn in regard to this point since the crop of bacteria for the individual fermentations was not determined. Only by a comparison of the energy available per unit organism could the minimum require-

TABLE 13

Energy balance of Cl. acetobutylicum on carbohydrates (31)

CARBOHYDRATE	GLUCOSE	MANNITOL	ARABINOSE
	millimoles	*millimoles*	*millimoles*
Total fermented	1000	1000	1000
Products formed:			
Acetic acid	100	275	220
Butyric acid	12	153	3
Acetone	313	78	394
Ethyl alcohol	288	60	247
Butyl alcohol	489	604	293
Carbon dioxide	2282	2070	1702
Hydrogen	1382	2140	946
Water	122	263	216
	kilogram-calories	*kilogram-calories*	*kilogram-calories*
$-\Delta H$ of substrate fermented	302.5	317.9	253.8
$\Sigma -\Delta H$ products × mole fraction of each	314.5	322.8	265.5
$-\Delta H$ of fermentation per mole substrate	12.0	4.9	11.7
$-\Delta F°$ of substrate fermented	219.0	226.1	184.4
$\Sigma -\Delta F°$ products × mole fraction of each	277.8	282.5	231.7
$-\Delta F°$ of fermentation per mole substrate	58.8	56.4	47.3

ments for bacterial activity be ascertained. A second point noticed in these data is that $-\Delta F°$ is two to three times as large as $-\Delta H$. This means that the organisms *may* have more energy available for work than is indicated by the heat of reaction. This additional energy would come from the environment. It must be emphasized that this does not mean that the organisms themselves possess the ability to convert heat into chemical energy, but only that the reactions taking place by reason of the activity

of the bacteria can proceed in a manner that will convert heat into free energy. Whether this actually occurs or not will depend upon the degree of reversibility of the system—the calculated values are the maximum free energy available and do not tell us how much of this is actually available. It is of interest to note that Burk (12) reports a similar finding for the lactic acid-glycogen system in the muscle.

III. THE ENERGETICS OF NITROGEN FIXATION BY BACTERIA

Nitrogen fixation by Azotobacter

The energy changes in the fixation of atmospheric nitrogen by bacteria have been the subject of much controversy. No doubt this is due to the lack of knowledge regarding the mechanism of the fixation as well as the contradictory nature of the data in regard to the carbon:nitrogen ratio. However, in the case of fixation of nitrogen by Azotobacter there is sufficient information available to hazard an estimate of the efficiency of this organism in its synthetic activity. Kostytschew (35) and his coworkers claim that Azotobacter fixes atmospheric nitrogen by reduction, i.e., ammonia is the first product formed, and offer some experimental evidence for this view. Linhart (41) calculated the efficiency of the organism to be about 1 per cent. He assumed that ammonium hydroxide was formed from nitrogen and water, but does not give the equation for its formation. The reaction is usually written,

$$1/2\ N_2 + 5/2\ H_2O \rightarrow NH_4OH(aq.) + 3/4\ O_2,$$

a synthesis that is very unlikely since $-\Delta F^\circ = -78.5$ kg-cal. Calculations show that with an equilibrium concentration of 10^{-6} moles of NH_4^+ and OH^- and a partial pressure of 0.2 atm. of O_2 a fugacity (pressure) of about 10^{45} atm. of nitrogen would be required. A second criticism is the fact that he used standard free energy values for all the reactions, a condition that obviously does not exist in the sphere of influence of the organism. Finally, the data used for the C:N ratio is misleading. While for practical purposes it may be considered that the energy used for nitrogen fixation is only about 1 per cent of that liberated in the com-

bustion of the carbohydrate, still this is not the problem with which we are concerned. There is no doubt but that a large amount of the energy that is set free by the organism in the burning of the carbon source is used for purposes other than nitrogen fixation. Energy for the latter must be differentiated from that used in cell maintenance, growth, work, etc. The work of Omeliansky (52), Koch and Seydel (34) and others indicate that in young cultures the C:N ratio is greatly decreased. Koch and Seydel in one series of experiments secured an average of 75 mg. nitrogen fixed per gram of carbohydrate used. If we consider only the energy that is involved in the fixation of nitrogen, it follows that under optimum conditions, the efficiency of Azotobacter is probably much higher than 1 per cent and very likely compares favorably with that of the autotrophic organisms. The work of Meyerhof and Burk (46) confirms this. By an application of thermodynamics to the probable reactions that take place in the fixation of nitrogen by Azotobacter, it was shown that 4.81 moles nitrogen should be fixed per mole of sugar used and that $\frac{\text{cc. } N_2 \text{ fixed}}{\text{cc. } O_2 \text{ used}}$ should have a maximal value of 0.915. Meyerhof and Burk studied the consumption of oxygen and the fixation of nitrogen by 24 to 48 hour cultures of Azotobacter over periods of 6 to 12 hours by the use of micro methods. With normal oxygen tension (0.21 atm.) the efficiency ratio (cc. N_2 fixed/cc. O_2 used) was found to be only 0.0025 to 0.008. However, with a change in the gas pressures, especially that of the oxygen, they found that this efficiency ratio could be increased to as high as 0.10. It appeared that the lower the oxygen tension, the higher is the efficiency, although the absolute amount of nitrogen fixed was markedly decreased if the oxygen tension was 0.01 atm. (5 per cent of normal) or lower. With an oxygen tension of 0.05 atm. (25 per cent of normal) the absolute fixation was increased two- to six-fold and an efficiency of 0.01 to 0.02 was obtained. These results indicate that under optimum conditions the "machine efficiency" of the organism with regard to nitrogen fixation varies from 2.5 to 10 per cent.

Burk (13, 14) has recently published results of further studies

on the relationship of the pressure of oxygen and nitrogen to the efficiency of nitrogen fixation by Azotobacter. He found that in an atmosphere containing 20 per cent oxygen, it was necessary to have at least 6 per cent nitrogen in order that fixation might take place. Up to 0.5 atm. (50 per cent) of nitrogen, the rate of growth (or fixation) was approximately directly proportional to the pressure of this gas. The maximum fixation occurred at 5 to 10 atm. of nitrogen. The presence of humic acid markedly increased the rate and efficiency of fixation and decreased the limiting nitrogen pressure to less than 1 per cent (0.01 atm.). If ammonia nitrogen were added in order to prevent the assimilation of elemental nitrogen, the stimulative action was still evident. Therefore, Burk concluded that the direct effect of humic acid was on the growth metabolism of the bacteria and not on the fixation. The efficiency of the fixation as measured by mg. nitrogen fixed/mg. glucose consumed was increased from 0.25 per cent to 0.72 per cent when the initial glucose concentration was decreased from 10 per cent to 1 per cent. Burk also used the ratios (1) K = count increase/total count ÷ respiration rate increase/initial respiration rate, and (2) cell number increase/respiration due to growth, as measures of the efficiency of the organism. With these criteria, he found a marked increase in efficiency as the pressure of oxygen was lowered below 0.05 atm. However, this increase with the lower pressures was independent of nitrogen fixation. Under all conditions, the efficiency of growth (or efficiency of fixation) was decidedly increased with an increase in the rate of growth (or rate of fixation). As a result of these findings, Burk concludes that: (*1*) the oxygen pressure functions of Azotobacter, with respect to growth, growth efficiency, respiration and humic acid, have nothing to do with the actual process of fixation; (*2*) the nitrogen pressure affects solely the fixation and not the general growth metabolism of the organism.

Burk (11b) in a discussion of the energetics of nitrogen fixation by Azotobacter concludes from these studies on growth and fixation efficiency that probably the first product of fixation occurs in a reaction requiring little, if any, energy. He points out that

if Azotobacter is grown in free or fixed nitrogen under conditions in which the rates of growth are equal, equal quantities of energy (supplied by the oxidation of glucose) are required to produce equal dry weights of the organisms. This means that the efficiencies of growth in free and fixed nitrogen are equal, and therefore, the energy consumption required for the overall process of fixation from nitrogen gas to ammonia (as distinguished from simply the first step) is small, or zero as far as the experiments are capable of deciding. Moreover under optimum conditions for efficient growth and fixation (very low oxygen tension, etc.) 1 gram of sugar will suffice to fix 0.1 gram of nitrogen and produce 1 gram of dry weight of growth. Since most aerobic organisms require 2 to 10 grams of carbohydrate to produce 1 gram of dry weight, it would appear likely that this 1 gram of sugar used by Azotobacter is required solely for growth purposes and practically none is used in the fixation. All the experiments showed that when Azotobacter is grown in air, its respiration is such that much more energy is available than is actually required for growth and maintenance, but none of this excess energy can be charged to nitrogen fixation since the surplus occurs whether or not nitrogen is being fixed. Burk shows in addition that the first step in the fixation concerns a compound that is in equilibrium with the nitrogen of the air. This key compound is formed instantaneously from free nitrogen as rapidly as it is used in growth, as the concentration present at all times is approximately proportional to the pressure of nitrogen up to 0.5 atm. Hence its formation is represented by an equilibrium reaction with high reversibility, which means that the free energy involved in its formation is substantially zero. The formation of this compound (without expenditure of energy) is fixation in the strictest sense of the term. Subsequent conversions of this initial or key compound into the Azotobacter cell might involve free energy changes that are irreversible and hence require some energy, but this possibility is presumably ruled out by the data presented earlier in the paragraph.

From a consideration of the foregoing discussed papers, it appears that the value assigned for the efficiency of Azotobacter

in the fixation of free nitrogen depends to a great extent upon experimental conditions and, of course, upon the definition of efficiency employed. In view of this variability, it appears to be the better part of valor to avoid a definite statement as to the efficiency of nitrogen fixation in nature by this organism. On the other hand, the progress made toward a solution of this problem by Meyerhof, Burk and their collaborators, makes it appear hopeful that the answer will be available in the near future.

Nitrogen fixation by Cl. pasteurianum

Little work has been done on the energetics of the fixation of nitrogen by the anaerobic species *Cl. pasteurianum*. This fixation is usually considered as one of very low efficiency, since the average value of nitrogen fixed per gram of carbohydrate used is 1 to 2 mg. However, aside from the question of what proportion of this energy is used in the actual fixation, the fact that the carbohydrate is only partially degraded must not be forgotten. For example, if glucose is broken down by this organism according to the equation,

$$C_6H_{12}O_6 \rightarrow 2\ CO_2 + C_3H_7COOH + 2\ H_2$$
$$-\Delta F^\circ = 64.5 \text{ kg-cal.}$$

$-\Delta F^\circ$ is less than one-tenth the corresponding value for the complete combustion of glucose to carbon dioxide and water brought about by the aerobic species Azotobacter (+685.8 kg-cal.). Neglecting, in the above reaction, the changes in $-\Delta F$ incident to change of concentration of the resultants and reactant, it may be readily seen that *Cl. pasteurianum* is as efficient, if not more so, in its utilization of available energy than is Azotobacter. The apparent inefficiency is due to the comparison of the nitrogen fixed to the carbohydrate that disappears, which ignores the butyric acid with its high energy content formed in the metabolism of this anaerobic organism. However it is by no means certain that the aerobic and anaerobic nitrogen fixers employ the same mechanism for the fixation. Because of the large quantities of hydrogen that are liberated in the destruction

of carbohydrate by anaerobic organisms of the Clostridium type, it seems probable that the fixation would take place thus:

$$1/2\ N_2\ (g) + 3/2\ H_2\ (g) \rightarrow NH_3\ (aq.)$$
$$-\Delta F^\circ = +\ 6.3\ \text{kg-cal.}$$

If this reaction occurs in the cell, the value of $-\Delta F$ would be changed according to the activities (concentrations) of the various substances concerned. On the basis of this equation Buchanan and Fulmer (10) have calculated the molality of the ammonia in equilibrium with 0.8 atm. of nitrogen and varying partial pressures of hydrogen. At a pressure of 10^{-4} atm. hydrogen (the partial pressure of hydrogen in the air according to Burk (11)) the equilibrium concentration of ammonia would be 3.69×10^{-2} M. Since the reaction takes place in the liquid phase, the fixed nitrogen would appear as ionized NH_4OH. In this case the pH of the cell would affect the equilibrium concentration of the NH_4^+. At neutrality, with a pressure of 0.8 atm. of nitrogen and 10^{-4} atm. of hydrogen, the NH_4^+ could reach a concentration of 11.9×10^{-1} M before the sign of the free energy change becomes positive. The equilibrium concentration increases tenfold for each unit change in pH toward the acid side and decreases tenfold for each unit change in pH toward the alkaline side. From these data it appears possible that the fixation of nitrogen by the anaerobic species may be exothermic. The energy derived from the utilization of carbohydrate is used for purposes other than nitrogen fixation.

Nitrogen fixation by Rhizobium

The study of the energetics of nitrogen fixation is even more restricted by lack of suitable and reliable data in the case of the symbiotic forms of bacteria, than it is in that of the free-living forms—Azotobacter and Clostridium. Since it has not been conclusively demonstrated that the nodule bacteria can fix nitrogen independent of the plant, the employment of experimental data gained in the few cases in which nitrogen was reported fixed in pure culture would be of doubtful value. In addition, the mechanism of fixation is by no means established. Studies of

nitrogen fixation in connection with the plant are complicated by the carbon and nitrogen metabolism of the latter, so that suitable technique has not been developed that would make possible a differentiation of energy used by the organism and that by the plant. If we reason by analogy that the fixation is similar to that of Azotobacter and the efficiency is of the same order, the conclusions reached are highly improbable. Thus Christiansen-Weniger (15) has shown that, for typical field experiments, the energy utilized for fixation would require a carbohydrate destruction of two to three times the total yield of the plants unless the process of fixation is much more efficient than is that of Azotobacter. He believes that the fixation must occur by an exother-

TABLE 14

Energy requirements for the fixation of nitrogen by legumes

Christiansen-Weniger

PLANT	TOTAL DRY WEIGHT		DIFFERENCE	NITROGEN FIXED	ENERGY REQUIRED FOR FIXATION OF 1 KILOGRAM NITROGEN
	Without nitrogen	With nitrogen			
	grams	*grams*	*grams*	*grams*	*kilogram-calories*
Bean	50.746	56.572	5.826	1.021	22,800
Alfalfa	60.659	68.487	7.828	1.184	28,884

mic process and offers experimental evidence in support of this. He found that the total dry weights of lupines, beans, and alfalfa grown in the presence of sodium nitrate showed only slight increases over inoculated plants grown in the absence of fixed nitrogen. From the small differences obtained, he calculated the energy utilized for fixation of the nitrogen on the basis of the equivalence of 1 gram dry plant material to 4.0 kg-cal. A typical set of results is given in table 14.

On the basis of these results, Christiansen-Weniger concludes that the amount of energy required in the fixation is so small that it is probable that this energy is used for the vital activity of the bacteria and that the actual fixation of nitrogen is accomplished by an exothermic reaction that yields energy to the

bacteria for their cell processes. However, the energy required for the fixation is not particularly small, as can be seen from the above table.

Rippel and Poschenrieder (56) take issue with Christiansen-Weniger in regard to this proposed autotrophic nature of the nodule bacteria. They observed an accumulation of starch in nodules while the plant was engaged in carbon assimilation which disappeared if the latter process was interfered with, as by transferring the plants to the dark. They regarded this accumulation of starch in the nodules as a means of supplying the needs of the bacteroids in fixing the nitrogen. They pointed out two sources of error in Christiansen-Weniger's work: (*1*) The nitrogen in the control was supplied as nitrate, which would require energy for reduction; (*2*) the difference in dry weight at the end of the experiment between the controls and plants that were inoculated is not necessarily a measure of the total carbon that was used in the fixation. It has been observed that the assimilation ability of the legumes for a given leaf area is twice that of other plants, and that if some of the leaves are prevented from assimilating carbon dioxide there is a compensatory increase on the part of the other leaves. Hence Rippel and Poschenrieder claim that the nodules could use a great deal more carbohydrate than was shown by Christiansen-Weniger's results and still have little influence on the final dry weight of the plant. Added evidence that carbohydrate is consumed in the nodules is furnished by the observation of Reinau that the "earth respiration" of legumes is much higher than that of mustard or of rye.

Rippel and Poschenrieder believe the hydrogen for the reduction of the nitrogen arises from a fermentation process similar to that supplying the hydrogen used in the production of alcohol by yeast. In this case, for every 180 grams of glucose used, 4 grams of hydrogen (fermentation) are produced. If this hydrogen were used entirely for reduction of nitrogen to ammonia, then a fixation of about 100 mg. per gram of sugar destroyed would result, which is of the same order as that fixed by young cultures of Azotobacter.

Burk (11) has made a theoretical study of the various mecha-

nisms suggested for the fixation of nitrogen by Rhizobium in the nodule of legumes and by the free-living forms, e.g., Azotobacter and *Cl. pasteurianum*. He stresses the point of view that the actual fixation in the primary compound is the question involved, and that subsequent reactions in which this first compound is concerned are a part of the nitrogen metabolism and for this reason the energy changes involved should be discriminated from those of fixation. By an application of thermodynamics to the various mechanisms proposed for the fixation, he shows that the fixation might take place by the reduction of nitrogen to ammonia, by oxidation to nitric acid, or by the formation of simple organic compounds and under certain conditions, all these mechanisms could yield energy and thus free the organism from the necessity of obtaining energy by the oxidation of carbohydrate. The sign of $-\Delta F$ of most of the proposed reactions for the mechanism is positive, provided the calculations are based on concentrations likely to be present in the cell rather than on the standard free energy equation. Since a number of these reactions require hydrogen, Burk suggests that this gas might be furnished by the small quantity in the atmosphere, 0.01 per cent, or by that arising from fermentation of carbohydrate by the cell. In the latter case, the energy loss suffered due to the hydrogen being liberated and not oxidized cannot be charged to nitrogen fixation since this liberation takes place irrespective of the fixation.

SUMMARY

The energy liberated in the metabolic processes of bacteria may be from two to one hundred times as much as is represented by the cells. Part of this is immediately converted into heat because of the irreversibility of the processes carried out by the organisms. A portion of the free energy available for work is used for cell growth and synthesis. Motion apparently requires only a small part of the total available energy. It appears that most of the free energy: (*1*) is transformed into surface energy; (*2*) performs osmotic work; (*3*) displaces equilibrium conditions within the cell; (*4*) possibly maintains oxidation-reduction potentials. Eventually the energy appears as heat.

The heat of reaction ($-\Delta H$) of fermentations carried out by different species of bacteria varies from 5 kg-cal. or less, to over 50 kg-cal. per mole of carbohydrate fermented. The standard free energy ($-\Delta F^\circ$) of a number of reactions studied is two to three times greater than the heat of reaction.

The energetics of nitrogen fixation by the aerobic species, Azotobacter, indicate that an efficiency of at least 10 per cent is reached under optimum conditions of fixation. The anaerobic species, *Cl. pasteurianium*, is equally efficient if energy liberated rather than carbohydrate used is taken as a criterion. It is probable that the actual fixation takes place exothermically by reduction of nitrogen under anaerobic conditions.

The data on the fixation of nitrogen by the legume bacteria in association with the plant suggest that the process is probably more efficient than with Azotobacter, and it is possible that the actual fixation occurs by an exothermic reaction.

In conclusion it is apparent that the field of bacterial energetics suffers from a lack of precise and adequate data. The results of Rubner and Tangl in the investigation of the general problem need to be checked by studies in which more modern technique is employed. The application of thermochemistry, calorimetry, and thermodynamics to a number of specific fermentations should yield valuable information for an understanding of these processes.

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