Energetics of the Pasteur Effect

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Under certain specified physico-chemical conditions the overall rate of biological phenomena is approximately proportional to the molar loss of free energy (molar entropy production). It is maintained as probable that these conditions are fulfilled in growing bacteria. On this basis it is shown that the devaluation of the energetic value of glucose by transition from aerobic to anaerobic growth will automatically cause a higher rate of glucose consumption, which is the Pasteur effect. An analogy between the energetic behaviour of the living cell and the AC transformer is indicated.

An experiment illustrating the relation between metabolism of growing and resting bacteria is described.

Pasteur has discovered¹ that yeast being able to utilize glucose both aerobically and anaerobically will do so with highly different rates. The anaerobic break-down of glucose, which may lead to lactic acid or to alcohol, is a much faster process than the aerobic break-down leading to carbon dioxide and water. This phenomenon which is shown by most cells having both aerobic and anaerobic metabolism is called the Pasteur effect. Various hypotheses have been advanced to explain the apparent inhibition of the anaerobic break-down (glycolysis or fermentation) by oxygen^{2,3}. In the present paper it is suggested that the rise in glucose consumption by transition from aerobic to anaerobic metabolism is a simple thermodynamic consequence of the accompanying devaluation of the energetic value of the glucose. No special regulatory mechanism seems to be necessary to explain the Pasteur effect. Contemporary ideas concerning the nature of transference of free energy from energy providing to energy requiring reactions provide a mechanism by which glucose consumption is automatically regulated by the energy requirements of the cell.

Before the demonstration of the energetics of the Pasteur effect it is necessary to have at hand two physico-chemical laws and one postulate:

1. The equivalence of loss of free energy and entropy production. This is an exact law.

2. The proportionality between rate of reaction and displacement from equilibrium. This is an approximate law which is valid near equilibrium only (generally for $-\Delta G \ll RT$).

3. A postulate of even distribution of entropy production amongst the vast variety of reactions taking place in a living cell.

We shall discuss these points in turn.

1. The majority of reactions in a cell are enzyme catalyzed reactions of the type

Examples of such reactions are dehydrogenations, transaminations and phosphorylations⁴. Is a the concentration of the species A, b of B, etc., we may write the condition of equilibrium

$$\frac{a \cdot b}{c \cdot d} \cdot K = 1$$

This condition is of course not obeyed by reactions going on in the metabolizing cell. If it were, the rate of the reaction considered would be zero. In general we have

$$\frac{a \cdot b}{c \cdot d} \cdot K = a$$

where $a > 1$ if the reaction is proceeding from left to right. This gives

$$-\Delta G = \Delta\mu = T\Delta S'' = RT \ln a$$

where $-\Delta G$ is the loss of free energy, $\Delta\mu$ the difference in chemical potential, and $\Delta S''$ the corresponding entropy production. This is, when motility and radiation is excluded, the only mechanism by which free energy dissipates from the cell. It is true, of course, that certain reactions apparently proceed against affinity, the free energy necessary being provided by some other reaction, but such reactions are now known to proceed in steps, each of which are enzyme catalyzed according to the principle outlined above⁴. The total loss of free energy in metabolism equals the integral entropy production.

Let us consider a bacterium growing on a medium with glucose as sole carbon source. The glucose is providing carbon for new cell material. The protein matter and other cell constituents will contain carbon which on average is slightly more reduced than glucose, so the free energy of combustion per carbon atom will be somewhat higher than in glucose. Accordingly, the

Fig. 1.

Ordinate: Oxygen consumption (mg/l), Abscissa: time (hours) 1: $C_{\text{NH}_4^+} = 5.10^{-3}$, 2: $C_{\text{NH}_4^+} = 1.3.10^{-4}$.

carbon dioxide production during aerobic growth is higher than the oxygen consumption. The necessary free energy will be provided by the reactions:

Some of this free energy is as indicated invested into the cell constituents but the main part will be degraded into heat and thus entropy produced as described above. Every reaction in the cell takes part in this entropy production. If some of the reactions are stopped, their contribution to the entropy production will disappear, and the consumption of free energy must accordingly decrease.

This is demonstrated by Fig. 1. Two flasks containing a dilute air-saturated glucose medium were inoculated with equal amounts of a suitable *Bacterium lactis aerogenes* culture. One of the flasks contained a small amount of nitrogen (ammonium sulphate) only, so growth would stop after a couple of hours. The contents of the flasks were distributed amongst a number of glass stoppered bottles which were filled up into the neck so they did not contain any gas phase when stoppered. The bottles were incubated in a water thermostat at 25° C, and at suitable intervals one of each series was taken out and oxygen determined according to Winkler. Fig. 1 shows that as long as there is nitrogen available for growth the behaviour of the two series is identical. When nitrogen

was used up in the series with limited supply, the rate of oxygen consumption fell to about 16 % of the value during growth. The reason is clearly that all reactions leading to synthesis of new cell material have now ceased, and so has the contribution of these reactions to the total entropy production of the cell. Consequently, the rate of loss of free energy, and therefore the rate of oxygen consumption, must decrease.

2. Prigogine⁵ and others have demonstrated that the rate of a chemical reaction proceeding with small affinity, *i.e.* near equilibrium, is proportional to the affinity. In this section we shall see that this law has an essentially better region of validity when simple enzyme reactions are considered. Let us take the reaction

a and b being stationary concentrations of the intermediate metabolic products considered. The reaction is catalyzed by an enzyme, and let the mechanism be

where X_1 and X_2 are enzyme and enzyme-substrate complex. It is generally assumed that the Michaelis equation

$$s = \frac{k \cdot E \cdot a}{K_m + a}$$

where $k = k_{-2}$, $K_m = \frac{k_{-1} + k_2}{k_1}$ and E the enzyme concentration is an adequate expression for the rate of such reactions. The assumption leading to this equation is that the first step is proceeding in both directions, the second only from left to right. This assumption can not be valid for the majority of reactions in the cell as it leads to a large displacement from equilibrium and therefore to a large entropy production per reaction, which, as we shall see in the next section, is not justified when the total loss of free energy is compared with the number of reactions going on in the cell. We must therefore take (-2) into consideration, and a steady state treatment gives:

$$s = E \frac{k_1 k_2 a - k_{-1} k_{-2} b}{k_1 a + k_{-1} + k_2 + k_{-2} b}$$

a_0 and b_0 being a set of equilibrium values of the concentrations of A and B gives:

$$k_1 k_2 a_0 = k_{-1} k_{-2} b_0$$

Keeping the concentration of B constant equal to b_0 and defining $\alpha = a/a_0$ gives:

$$s = E \frac{k_1 k_2 a_0 (\alpha - 1)}{k_1 k_0 \alpha + k_{-1} + k_2 + k_{-2} b_0}$$

α has the same thermodynamic significance as in the preceding section. Christiansen ⁶ has shown that in the denominator of such expressions the terms will generally be of very different order of magnitude. Assuming symmetry in A and B we have either

$$k_1 a_0 \sim k_{-2} b_0 \gg k_{-1} \sim k_2$$

or

$$k_1 a_0 \sim k_{-2} b_0 \ll k_{-1} \sim k_2,$$

Now it is well known that the rate of bacterial growth in synthetic media is generally independent of concentrations of medium constituents within very wide limits. This indicates that the rate of enzyme reactions does not change very much when concentrations of the reacting molecules are changed, so numerator and denominator would probably be influenced in the same way by change in concentrations. This rules out the second inequality, so we have

$$s = E \frac{k_1 k_2 a_0 (\alpha - 1)}{k_1 a_0 \alpha + k_{-2} b_0}$$

Fig. 2 shows the relation between s/s_0 and α , where s_0 is the limiting rate for $\alpha \rightarrow \infty$, when $k_1 a_0 = k_{-2} b_0$. Fig. 3 shows the corresponding relationship between s/s_0 and $\ln \alpha$. The function plotted is

$$s/s_0 = \frac{\alpha - 1}{\alpha + 1}$$

It is easily seen that this function is identical with

$$s/s_0 = \tanh(\frac{1}{2} \ln \alpha)$$

This rough treatment shows that the law

$$s = EX \ln \alpha = EX(\Delta G)/RT$$

where

$$X = k_1 k_2 a_0 / (k_1 a_0 + k_{-2} b_0)$$

Fig. 2.

is reasonably valid up to about $\ln \alpha \sim 1.7$ or $\Delta G = 1$ kcal. The corresponding rate is about 70 % of the limiting rate. This agreement is far better than the agreement calculated from simple noncatalytic reactions⁵. The reasons are two: Firstly our reaction proceeds in two steps amongst which the loss of free energy is distributed, and secondly the competition of A and B for the enzyme gives a rate expression which favours the agreement.

The reaction here investigated is an oversimplification of the typical cell reaction treated in the preceding section. A similar treatment of the more general reaction encounters two difficulties: The mechanism of such reactions is as yet not exactly known, and if it were, the mathematical treatment would be extremely complicated. The result would presumably be less unique than outlined above, although the general features leading to the validity of the rate-affinity law are retained. A possible source of complication is the feature that the equilibrium

has three "degrees of freedom" where

has one only. It is seen that in the simple case it is indifferent which equilibrium is taken as reference state as long as the concentrations of A and B are not very small. As the number of steps in the more general enzyme reaction is

Fig. 3.

necessarily greater than two an even better region of validity might be expected. The fact that growth rate of bacteria is largely independent of concentrations of medium constituents supports the assumption of validity in the more complex case also.

We shall leave this section with the remark that the treatment of the relation between affinity and rate of enzyme reactions should be taken as an introduction to a general treatment which is in progress.

3. In this section we shall compare the total loss of free energy with the number of reactions going on in the cell. Determination of the respiration of growing bacteria shows that only a fairly small fraction of the glucose turnover is oxidized. For *Bacterium lactis aerogenes* this fraction is, according to investigations to be published later, about 1/4. The loss of free energy per mole glucose oxidized is about 700 kcals. A part of this free energy is invested in the carbon atoms of the cell material, so the total loss of free energy per mole glucose metabolized aerobically will probably be of the order 150 kcals. The fate of a glucose molecule oxidized is essentially as follows:

Glucose is through about 13 steps, the glycolytic enzyme system, converted into two molecules of pyruvic acid. After about half of these steps the glucose is split into two triose molecules, each of which must go through the last part of the sequence, so there has effectively been about 20 steps. Now pyruvic acid is oxidized to carbon dioxide in the tricarboxylic acid cycle, a number of

coenzyme molecules at the same time being reduced. It is well known that free energy is trapped out of the glycolytic reactions by the reaction

(ADP = Adenosinediphosphate, ATP = Adenosinetriphosphate)

which proceeds against affinity^{4,7}. There is every reason to believe that exactly the same mechanism is effective when free energy is trapped out of other energy providing reactions in cell metabolism. The tricarboxylic acid cycle is known to comprise some ten steps when phosphorylations are not considered. It is also known that a part of the free energy lost during these steps is made available to the cell, so we must necessarily assume that the reactions in this cycle include a number of as yet unknown phosphorylations and transphosphorylations. If all the known reactions provide energy, we must have three steps, *viz.* phosphate uptake before the reaction, and phosphate transference afterwards, for each reaction, or thirty steps altogether. There were two molecules of pyruvic acid, so the score is now, when glucose is oxidized to carbon dioxide, and 12 coenzyme molecules are reduced, 80 enzyme reactions of the type considered in section 1. The coenzyme molecules are re-oxidized in reactions with the flavoproteins, and through one-electron reactions, 24 electrons per glucose molecule oxidized, the hydrogen is transferred to oxygen through an unknown number of steps catalyzed by cytochromes and cytochrome oxidase. Some of the free energy lost in these steps is undoubtedly trapped out into the ADP-ATP system. The number of steps must be quite considerably. Even a small number like 5 per hydrogen atom would lead to a total of more than 200 reactions per mole glucose oxidized. It is not unlikely that the average number of steps per glucose molecule incorporated into new cell material is very similar. Most of the carbon compounds in the cell have very little structural similarity with glucose indeed. So the result of this discussion is that the average number of steps per glucose molecule metabolized is at least of the same order as the average number of kcals free energy lost through irreversibility. That means that the average loss of free energy is compatible with the law of proportionality between rate and displacement from equilibrium. The question is now whether the total loss of free energy is distributed evenly amongst the reactions of the cell. The only way we can go here is to postulate that this is the case.

This postulate gains in credibility when it is realized that it is equivalent with the assumption that the total amount of protein of the cell is distributed adequately on the various enzyme functions. It is easy to prove that if a certain amount of protein is at disposal for the formation of a number of enzymes catalyzing a sequence of reactions, then there will be a certain distribu-

tion which gives higher overall rate of the sequence than all other distributions. Such an enzyme system would be completely without "bottle necks".

The postulate here outlined should of course be taken with some reservation. Work to investigate its validity is in progress. In favour of the postulate is maybe the idea⁸ that a large loss of free energy in a single enzyme reaction might be fatal to the enzyme. Further, the adaptation theory^{9,10} of bacterial training, which has in some cases been proved to be definitely superior to the mutation theory, shows that when bottle necks are introduced into the enzyme machinery by addition of drugs to growth media, or by transference to media with new carbon sources, then the bacteria are able to remove these bottle necks by expansion of the enzymes, the activity of which were reduced by the drug, or which became necessary in greater amounts by a new mode of metabolism. The adaptation theory clearly supports the idea that growing bacteria endeavour a bottle neck free composition.

The arguments leading to low average affinity for enzyme reactions and therefore to proportionality between rate and loss of free energy may be extended as follows: Bacteria are able to grow logarithmically on a variety of different media with highly different growth rates^{9,11}. Thus *Bacterium lactis aerogenes* will grow on a synthetic medium with glucose as sole carbon source at 40° C with a mean generation time (MGT) of 33 minutes. If this organism is transferred to a broth medium, the MGT will fall to about 18 minutes, *i.e.* the growth rate is almost doubled. The reason is, according to the points of view here advanced, that the broth medium contains a large number of ready made cell constituents, which the cell is saved to synthesize. That means that no free energy will be lost on the reactions normally leading to these substances, so more free energy is available to reactions which are still necessary. Such reactions will be displaced further from equilibrium, and therefore proceed with higher rate. The rate of these reactions will of course in the broth medium be lower than the limiting value for infinitely high affinity, and the rate in the synthetic medium will thus be lower than about half this rate. That will, if affinity is evenly distributed, be equally valid for all other reactions. Inspection of Fig. 3 shows that this is well within the region where the rate-affinity law should be applicable. We shall in the following discussion of the mechanism of the Pasteur effect assume that affinity is fairly evenly distributed, *i.e.* the rate of enzyme reactions is essentially proportional to the loss of free energy.

We shall now consider the fate of the free energy lost when glucose is converted aerobically to the pyruvic acid-lactic acid state:

$$-\Delta G = 60 \text{ kcals}$$

The exact value will of course depend somewhat on the concentrations of glucose and pyruvic acid, but is rather insensitive. A change by a factor 1 000 will only change $-\Delta G$ by about 4 kcals. $-\Delta G$ may be split up into two parts:

$$-\Delta G = m + n$$

where m is the free energy trapped out by the reaction

m depends on two factors: the number of times this reactions goes on, determined by the stoichiometry of the glycolysis sequence, and the difference in chemical potential between the two sides. The building up of this chemical potential is the combined effort of all reactions providing free energy, and a steady state is created, also controlled by the processes utilizing free energy by the reversal of the reaction. The rest of the free energy, n , is lost inside the sequence, a corresponding amount of entropy simultaneously produced. This part of the free energy loss is the driving force of these reactions, and the rate of glucose utilization is according to the view advanced above proportional to n . Let the oxygen disappear. The contribution to the ATP pool from all reactions below glycolysis is ceased. ATP is in the first instance used with the same rate but the ATP concentration very soon drops, and a new steady state is created, in which the chemical potential between the two sides of the energy trapping reaction is reduced by a certain factor, *i.e.* the value of m is decreased. The inevitable consequence is that n increases, and so does the rate of attack on glucose which is proportional to n . This means that the devaluation of the energetic value of glucose caused by absence of oxygen is automatically compensated by a higher rate of glucose-consumption, which is the Pasteur effect. The rate of growth is controlled by the energetic value of ATP, and is accordingly reduced during anaerobiosis¹².

The corresponding mechanism of the phenomenon shown in Fig. 1 is of course: When growth stops because nitrogen is used up, a great number of reactions using ATP will cease and ATP will accumulate. The chemical potential between the two sides of the energy conserving reaction will increase, *i.e.* m will increase. Hence n will decrease, hence a much slower attack on glucose.

In this elucidation of the mechanism of the Pasteur effect much attention has been placed on the ADP-ATP system. The author does not claim any originality in the assumption that the glucose consumption "hangs on the ATP/ADP ratio", but he does claim that only the physico-chemical development given here justifies to explain the Pasteur effect by this assumption.

The most important feature of the treatment is that no special regulatory mechanism is necessary to explain the effect. Indeed, it might be said that if living cells able to utilize glucose both aerobically and anaerobically did not show the Pasteur effect, then there would be something fundamentally wrong in the current ideas concerning the structure of biochemistry. It should be emphasized that the attention paid to the ADP-ATP system is partly a matter of convenience in the perspicuous demonstration of the energetic mechanism. The real significance should be concentrated on the fact that the entropy production corresponding to the loss of free energy in respiration or glycolysis is distributed over all reactions going on in the cell, and the assumption that the energy is trapped out stepwise by some obligatory mechanism more or less common to all energy providing steps. All systems working in accordance with this principle will display a "Pasteur effect".

An interesting analogy to the energetic behaviour of the living cell is shown by the AC transformer. The loss of energy in the primary side is almost completely determined by the composition of the secondary circuit. It is possible to keep the state in the secondary circuit unchanged when the tension on the primary side is altered, provided the ratio of the transformer is changed simultaneously. Under such circumstances the product of tension and current, *i.e.* the loss of free energy, will remain constant. That the integral energetic value of aerobic and anaerobic utilization of glucose is also of the same order is seen from the following rough calculation: When 1 mole of glucose is converted aerobically into *Bacterium lactis aerogenes*, 1/4 is oxidized, the corresponding loss of free energy being $0.25 \times 700 = 175$ kcals. The rest of the glucose, 0.75 mole, is used for other purposes, essentially for growth. To get the same amount of bacteria anaerobically 3.5 mole of glucose is needed¹². Of this amount 0.75 mole is invested in the same way as before, and 2.75 mole is converted into lactic acid, the loss of free energy being $2.75 \times 60 = 165$ kcals. The entropy production is not distributed in the same way between the primary and the secondary side of the chemical transformer in the two cases.

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