THE RELATIONSHIP BETWEEN FERMENTATION AND ENZYMATIC ADAPTATION*

by

CATHERINE B. FOWLER**

Service de Physiologie Microbienne, Institut Pasteur, Paris (France)

The present paper reports on studies of the metabolism of cultures of *Escherichia coli* transferred from aerobiosis to anaerobiosis. We have found changes in growth and increase in the utilization of glucose which suggest that the anaerobic utilization of glucose requires the synthesis of an adaptive enzymatic system.

MATERIALS AND METHODS

E. coli (strain ML) was used for these experiments. This strain grows well in a synthetic medium with a division cycle of about 60 minutes at 37°. The composition of this synthetic medium was as follows:

$(NH_4)_2SO_4$	2.0	grams per liter
$MgSO_4.7H_2O$	0.2	
$CaCl_2$	0.01	
$FeSO_4.7H_2O$	0.0005	
$CaCl_2$	0.0001	
$MnCl_2$	0.001	
$ZnSO_4$	0.0001	
$CuSO_4$	0.00001	
$MoO_4(NH_4)_2$	0.0001	

For the studies on growth this medium was buffered at p_H 7.4 with 0.1 *M* phosphate and supplemented with glucose or other carbon source at 4 mg/cm³.

Growth was estimated by measuring the optical density of the cultures in the Meunier nephelometer. For *E. coli* ML in the exponential phase of growth under the conditions of measurement, 1 unit of optical density corresponds to approximately 10^6 bacteria/ml.

All the experiments were made at 37°.

1. *Studies on the growth of a culture passed from aerobiosis to anaerobiosis*

The initial experiment was the study of growth of a culture passed from aerobiosis to anaerobiosis.

The cultures were made in T-tubes[1] suitable for gentle shaking, for measurement of optical density and for flushing with a gas. The synthetic medium containing glucose as the carbon source was inoculated and divided among three T-tubes. The tubes were shaken at 37° at a rate to assure optimal aeration. When the cultures were well within the exponential phase of growth, one of them remained as control and the two others were flushed with nitrogen (purified of traces of oxygen by slow passage over hot copper). The tubes were then sealed with paraffin in order to prevent air from leaking in.

A typical growth curve is shown in Fig. 1. The optical density is expressed as its logarithm to the base 2, a convention after MONOD[2] which facilitates the evaluation of

* Travail effectué avec l'aide d'une subvention du National Institute of Health des Etats-Unis d'Amérique.

** Boursière du Ministère des Affaires Etrangères, Service des Relations Culturelles.

References p. 573.

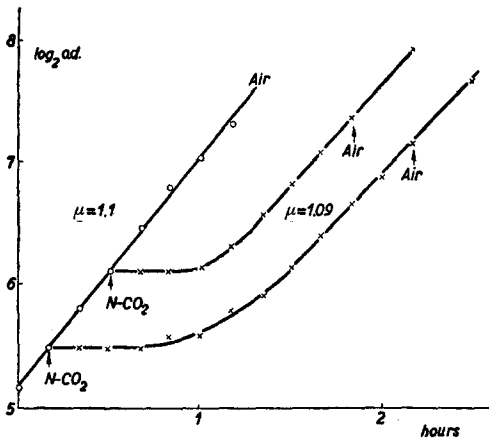

Fig. 1. Growth curve of *E. coli* transferred from air to nitrogen synthetic medium with glucose at 4 mg/ml. Temp. 37°. μ = growth rate expressed in number of doublings per hour

growth rates. It is seen that the growth stops completely as soon as the culture is removed from air and does not start again until after a significant latent period. The culture then begins to grow with an increasing rate until the growth rate is the same, or very nearly the same, as under aerobic conditions. If then the tubes are opened to air, there is no change in growth rate.

This experiment has been repeated many times under varying conditions. It has been found that the period during which there is no growth varies from 30–50 minutes, and that by 70 minutes the exponential rate has again been re-established. The length of the latent period is independent of the concentration of glucose, and independent also of p_H between 6.0 and 8.5. Supplementing the medium with caseinhydrolysate shortens the latent period to 10–20 minutes. These same changes in growth when a culture is transferred to nitrogen were found when the culture had been growing on sources of carbon other than glucose: lactose, fructose and pyruvate. When maltose was the carbon source, growth did not resume under anaerobic conditions until after many hours.

We have observed some variation in the length of the latent period and the anaerobic growth rate with various sources of carbon. However, in no case, has it been observed that the growth continues uninterrupted when the culture is passed to anaerobiosis. It is this fact which merits our consideration.

The anaerobic growth is re-established after a latent period too short to represent a selection. The shape of the growth-curves reminds one of the phenomenon of "diauxie" as described by MONOD[3]; a culture which has been grown on glucose does not grow immediately when certain other sugars are substituted as the carbon source. Growth resumes only after a period of time during which the enzyme specific for the attack of this second sugar is synthesized. In the present case, glucose is the only source of carbon under both the aerobic and anaerobic conditions of growth. The fact that it stops, and is resumed only gradually after O_2 has been removed, suggests that the glycolytic system is gradually built up when the oxidative pathway is blocked by the absence of air. The term "glycolytic system" should be understood to mean the enzymes, transporters, and metabolites involved. The problem is, which elements of the system are implicated in this building up process. We considered it probable that one or several of the enzymes themselves were involved, *i.e.* that the building up process in question involved enzymatic adaptation "sensu stricto".

The following experiments were undertaken to test this hypothesis.

2. *Utilization of glucose during anaerobiosis*

Utilization of glucose by a culture passed into nitrogen after growth in air was evaluated by determination of glucose taken up from the medium and by measuring CO_2 production in a medium buffered with bicarbonate.

References p. 573.

A culture of *E. coli* growing aerobically in the synthetic medium supplemented with glucose at about 10 μM/ml was flushed with nitrogen. The culture was kept in a cylinder provided with an outlet at the bottom, and there was a continuous stream of nitrogen through the culture in order to allow the taking of aliquots without admitting air. Aliquots were taken every ten minutes and transferred to a boiling water-bath for 3 minutes. The cells were then centrifuged. Glucose was determined by the method of SOMOGYI with the modification of NELSON. Table I gives the uptake of glucose and the Q-glucose, or uptake during ten minutes by 1 ml of a culture at an optical density of 1000. In calculating the Q-glucose, the mean optical density for the ten minute period was used.

TABLE I

GLUCOSE UPTAKE OF *E. coli* DURING ANAEROBIOSIS

Minutes after culture is passed to anaerobiosis	Optical density	Glucose left	Glucose used	Q-Glucose*
0	512	11.3	0.0	—
10	510	11.4	0.0	—
20	511	11.4	0.0	—
30	510	11.4	0.0	—
40	540	10.6	0.68	1.31
50	588	9.8	1.58	1.58
60	640	8.8	2.61	1.68
70	705	7.5	3.88	1.88
80	775	6.1	5.20	1.77
100	960	3.1	8.50	1.79

* μM of glucose per 10 minutes per ml of culture of an optical density of 1000.

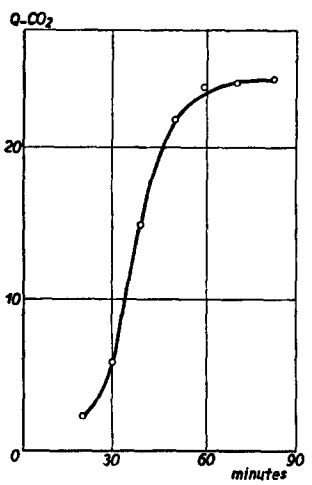

Fig. 2. Increase in fermentation during the first 90 minutes of anaerobiosis. Q–CO_2 = μl CO_2/10 min/ml of optical density 100

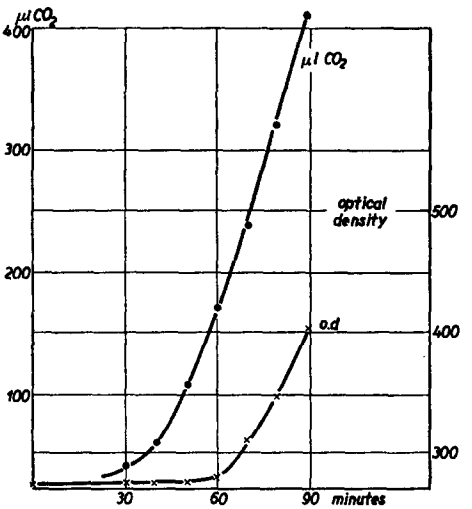

Fig. 2A. Evolution of CO_2 in NCO_3 medium, pH 7.4 and increase in optical density of a culture of *E. coli* during the first 90 minutes of anaerobiosis

This experiment was modified in order to determine the total final products of fermentation. The culture was grown in the synthetic medium buffered with bicarbonate at a concentration to give pH 7.5 under an atmosphere of 5% CO_2–95% N_2 (0.03 M). This medium contained only 0.2 mg/ml of phosphate and 2 mg/ml of ammonium sulfate. These concentrations are sufficient for the growth during the experiment and do not cause a significant retention of CO_2. The culture was grown aerobically under an atmosphere of 5% CO_2-air. Aliquots were pipetted into six Warburg vessels and the

References p. 573.

vessels flushed with 5% CO_2–95% N_2. CO_2 evolution was followed, while at intervals one of the vessels was withdrawn for measurement of optical density. The results of such an experiment are represented in Fig. 2. The Q–CO_2 represents the μl CO_2 per ten minutes per ml of a culture of optical density 100.

Because of the time required for flushing and equilibration, this method does not permit an evaluation of the CO_2 production during the first 20 minutes of anaerobiosis. But the method was used because it gives the fermentation of a suspension under conditions allowing exponential growth at maximum rate (that is to say, so far as we know, under "physiological" conditions), thus avoiding injurious effects resulting from centrifuging and washing.

The sudden increase in the final products of fermentation of about ten times of an initial very low value agrees with the abrupt uptake of glucose which was found. These results indicate that the cessation of growth of a culture passed from aerobiosis into anaerobiosis is the result of an inability to utilize glucose.

3. *The effect of 2–4 dinitrophenol*

MONOD[4] has shown that 2-4-dinitrophenol (D.N.P.) inhibits enzymatic adaptation. It was of interest to study the effect of D.N.P. on a culture passed into anaerobiosis.

Fermentation was measured as in the previous experiment except that the aerobic culture was centrifuged and resuspended in the same medium without glucose before it was passed into anaerobiosis. In this way CO_2 production could be followed from the time that glucose was added. Cells which have been centrifuged seldom adapt to the same extent as the uncentrifuged cells. The anaerobic growth rate is usually slower and the CO_2 production does not increase more than 5–6 times although the Q-glucose may be higher than the Q-glucose of an uncentrifuged cultures.

2 ml of the suspension were pipetted into a series of Warburg vessels with two side arms and flushed with 5% CO_2–95% N for 6 minutes. After equilibration glucose was tipped in from one of the side arms. Fermentation was followed and optical density was determined on one of the vessels at intervals. D.N.P. in a bicarbonate buffer at p_H 7.5 was tipped in from the other side arm at a final concentration of $2 \cdot 10^{-3}$ *M* at 0, 40 and 80 minutes after the addition of glucose. The results are shown in Fig. 3.

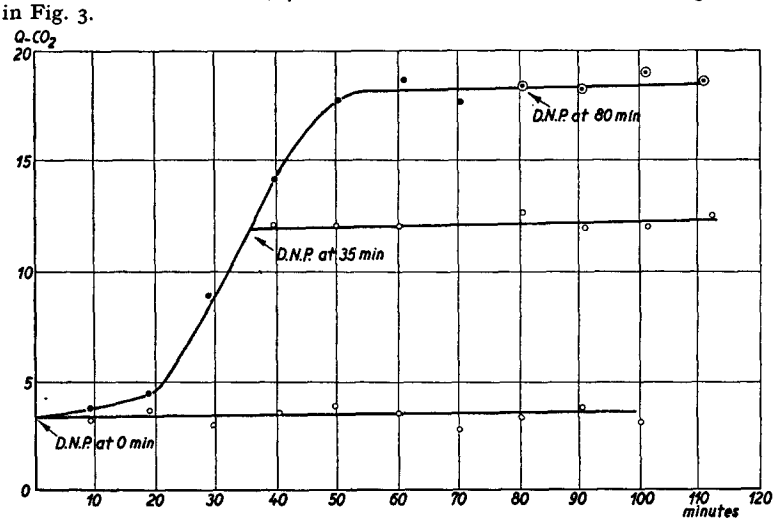

Fig. 3. Inhibitory effect of $2 \cdot 10^{-3}$ *M* D.N.P. on increase in fermentative power of *E. coli* transferred to nitrogen

References p. 573.

It is seen that the addition of D.N.P. stabilizes the $Q\text{-}CO_2$ at the level it had reached when the D.N.P. was added. At this concentration it completely inhibits any increase in fermentation when added at the same time as the glucose whereas it is without effect on the fermentation rate if added after it has reached a maximum. Lower concentration of D.N.P. inhibit only partially the increase in fermentative activity.

4. *Infection by bacteriophage*

MONOD AND WOLLMAN[5] have shown that infection by bacteriophage prevents *E. coli* from adapting to a variety of substrates. On the other hand, COHEN[6] has observed that bacteriophage does not reproduce in bacterial suspensions which were put under anaerobic conditions shortly after infection.

If fermentation is effected by an adaptive enzymatic system, and if it should be possible to show that infection with bacteriophage prevents adaptation to anaerobiosis the observation of COHEN might readily be explained. The experiments of MONOD AND WOLLMAN were repeated except that instead of passing a culture from glucose to an adaptive substrate, they were passed from air to nitrogen. For complete details of the experiment, reference should be made to the original paper already cited[5]. The following is a resumé of the proceedure followed.

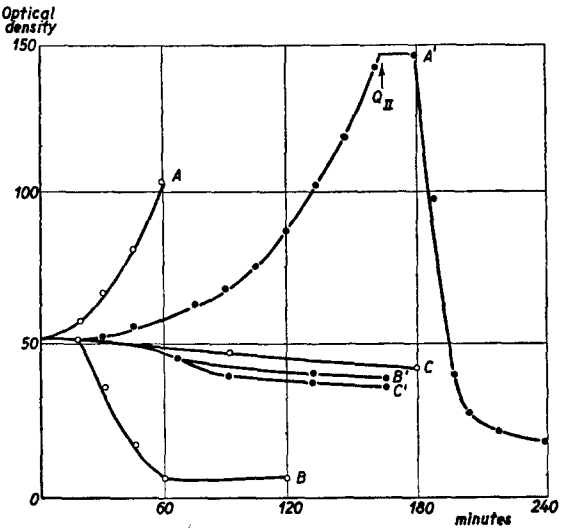

Fig. 4. Changes in optical density of aerobic and anaerobic cultures of *E. coli* B infected or uninfected with phage φ_{II}. Arrow shows time at which φ_{II} was added in control A'

A aerobic glucose no phage
B aerobic glucose phage
C aerobic no glucose no phage
A' anaerobic glucose no phage
B' anaerobic glucose phage
C' anaerobic no glucose no phage

E. coli B in a synthetic medium was shaken for 14–18 hours at 37° after growth had stopped because of exhaustion of glucose. The culture was diluted with fresh medium without glucose to an optical density corresponding to $5 \cdot 10^7$ bacteria/ml and divided between 6 T-tubes. Three of these tubes were kept in air and set up as follows:

A. Glucose (no phage)
B. Glucose + phage
C. No glucose + phage

Three other tubes were flushed with nitrogen for five minutes and then made up in the same way (A' B' C') and kept in the absence of air. The phage (φ_{II}) was added to give a final concentration of $5 \cdot 10^8$/ml in order to assure infection of all of the bacteria. The results are shown in Fig. 4.

In the aerated culture with glucose, growth started immediately (A). In the infected culture in the presence of glucose (B) there was no growth, and a sudden and rapid lysis occured at 18 minutes, which is the latent period characteristic for this phage. In the absence of glucose (C), the optical density decreased only slightly without production of bacteriophage.

The anaerobic cultures behave quite differently. In the presence of glucose, growth did not start until after a considerable latent period (A'). The culture infected at the beginning of the anaerobiosis in the presence of glucose (B') behaved exactly as the culture infected in the absence of glucose (C'). In both cases, the slight lysis which

References p. 573.

occured during the course of several hours did not represent a production of bacteriophage as shown by titrations of the two cultures. On the other hand, if the infection is made after the anaerobic growth rate is well established, lysis ensues after 18 minutes as under aerobic conditions. The production of phage per bacteria in the two cultures was found to be comparable.

Estimations of glucose showed that there is no measurable uptake of glucose in the culture infected within the first 5 minutes of anaerobiosis even after several hours. When the infection is made after anaerobic growth the glucose continues to be taken up until the culture is completely lysed.

These results indicate that a culture which has grown under anaerobic conditions is physiologically different from a culture just passed into anaerobiosis in that it can support the synthesis of bacteriophage in the absence of oxygen. The inability to do so during the first few minutes of anaerobiosis would seem to be due to the inability to utilise the carbon source. At the same time, infection during this period prevents adaptation to anaerobiosis in the same way that it prevents adaptation to certain substrates.

5. *The effect of exogenous nitrogen*

All of the experiments which have been described were made with suspensions of bacteria in the presence of ammonium sulphate. The centrifuged unwashed cells when resuspended in the absence of ammonium sulphate showed some increase in fermentation which was always followed by a rapid loss of the fermentation activity. However certain results suggested that a source of nitrogen was needed not only for the production but also for the maintenance of the activity. The following experiment was designed to clarify this problem.

A culture of *E. coli* growing aerobically was centrifuged and washed in the synthetic medium without ammonium sulfate. A suspension was made in this medium buffered with bicarbonate and aliquots were pipetted into 11 double side-armed Warburg vessels. After equilibration glucose was added to all the aliquots, and ammonium sulphate was added to give a final concentration of 2 γ N/ml in one series of five vessels and 4 γ N/ml in the second series of five vessels. The remaining vessel to which no ammonium sulphate had been added served as has been described. With these washed suspensions, the adaptation was very slow and the total growth was only 8% higher in the presence of 20 /γml of ammonium sulphate than in the presence of 10 γ/ml.

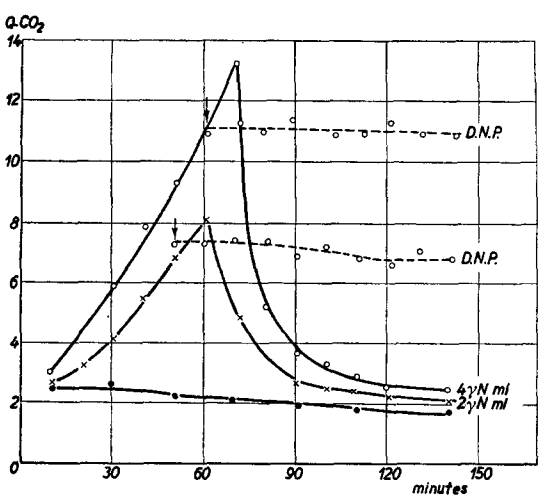

Fig. 5. The disappearance of fermentative activity due to the exhaustion of exogenous nitrogen and its inhibition by D.N.P. at $2 \cdot 10^{-3}$ *M*

Fig. 5 shows the results of this experiment. It is seen that the Q–CO_2 increases almost proportionally to the amount of exogenous nitrogen present and then falls abruptly to the unadapted level. With some suspensions, we have observed a decrease of as much as 80%–90% of the total acquired activity in the first ten minutes. In one vessel of each series, D.N.P. was added ten

References p. 573.

minutes before the Q–CO_2 had reached the critical level. In the presence of D.N.P. at 10^{-3} *M*, the Q–CO_2 did not fall but remained constant for more than an hour.

It is unlikely that D.N.P. protects against the loss of activity occuring in the absence of exogenous nitrogen by serving as a source of nitrogen. It is known to inhibit nitrogen uptake by bacteria *cf.*[7] and yeast[8]. Also, as was shown above, under similar conditions it can inhibit completely any increase in fermentative activity. The effect of D.N.P. in this system is quite comparable to the effect observed by SPIEGELMAN *et al.*[9] in certain yeast adaptations. These workers showed that D.N.P. stabilizes adaptation at a given level inhibiting both further increase or decrease in adaptive activity.

6. *The disappearance of fermentation in the presence of air*

Since the absence of air provokes an increase in fermentation, it was of interest to study the effect of air on a culture possessing a high fermenting power after anaerobic growth. This problem was approached in two ways.

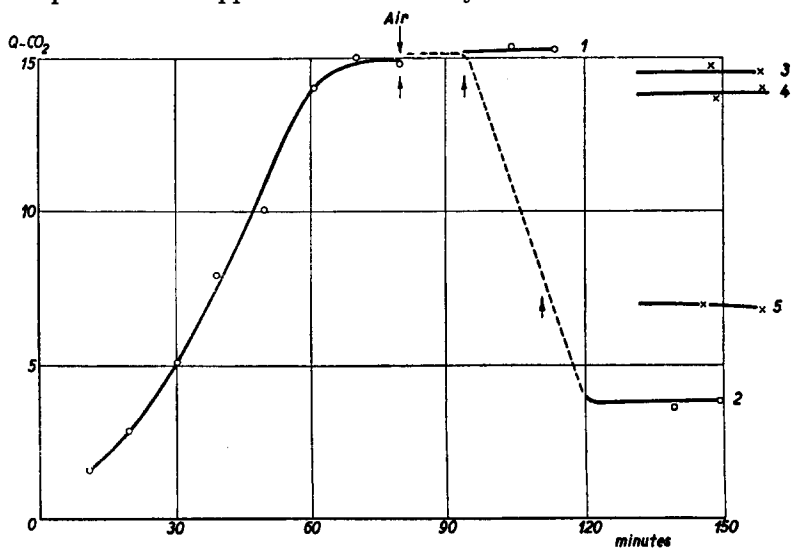

Fig. 6. The disappearance of fermentative activity during aeration of an anaerobically adapted culture and the protective effect of D.N.P.

The disappearance of the fermentative capacity in the presence of air was determined by following the fall in Q–CO_2 during aeration of a suspension which had been previously adapted to anaerobiosis.

2 ml of a suspension of *E. coli* in a synthetic bicarbonate medium were pipetted into a series of Warburg vessels and flushed with 5% CO_2-N. After equilibration the glucose was tipped in and the increase in fermentation was followed. The Q–CO_2 were derived as has been described. When the adaptation was complete, as indicated by a constant Q–CO_2, (see Fig. 6) the remaining 5 vessels were opened and shaken in air-5% CO_2. The fermentative capacity of the cells after 15 minutes (vessel 1) and after 40 minutes (vessel 2) of aeration were determined in the following way: 1.0 ml was removed from the vessel and the optical density determined. The vessel was then closed, flushed with 5% CO_2-N and the fermentation followed for 20 minutes. At the end of this time, the optical density was determined and the Q–CO_2 for the period derived.

The derived Q–CO_2 are shown in Fig. 6. From Fig. 6, it is seen that after 15 minutes of aeration (vessel 1), the Q–CO_2 is the same as the adapted anaerobically growing

References p. 573.

culture. However, after 40 minutes of aeration the fermentative capacity has been almost completely lost and the Q–CO_2 is that of an unadapted culture (vessel 2).

To the three remaining vessels, D.N.P. was added, at the time the vessels were opened to air (vessel 3) and after 15 minutes and 30 minutes of aeration (vessel 4 and 5). The optical density was taken at the time the D.N.P. was added. The D.N.P. was at a final concentration of 10^{-3} *M* which completely inhibits growth. After the total 50 minutes of aeration, the three vessels were closed, flushed with 5% CO_2–N and the Q–CO_2 determined. It is seen (Fig. 6) that D.N.P., added at the moment the vessel is opened to air, protects against the decrease in Q–CO_2 which would occur during the following 50 minutes of aeration. Thus the Q–CO_2 in the presence of D.N.P. and air for 50 minutes (vessel 3) is the same as for the anaerobically adapted culture, whereas the Q–CO_2 of the culture aerated in the absence of D.N.P. has fallen to the unadapted level

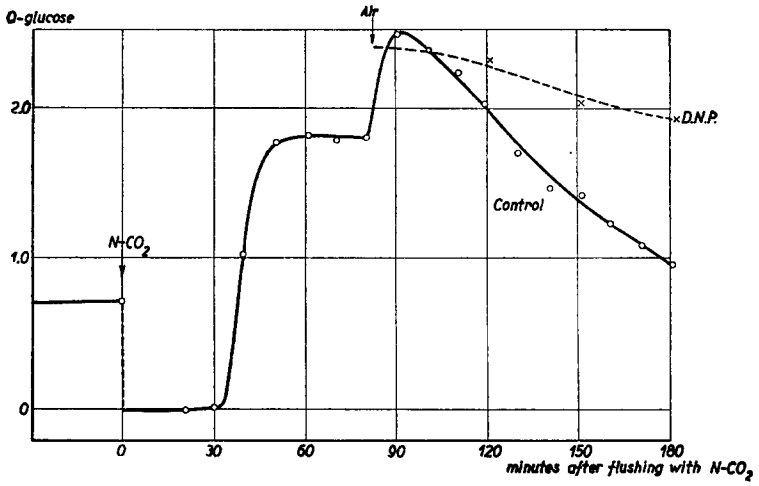

Fig. 7. The decrease in glucose utilization during aeration of an anaerobically adapted culture and the protective effect of D.N.P. at $5 \cdot 10^{-4}$ *M*

(vessel 2). The suspension which had shaken 30 minutes in air before the addition of D.N.P. and had then been aerated for an additional 20 minutes, maintained an intermediate Q–CO_2 which interpolated well on to the de-adaptation curve.

The decrease in fermentative power in the presence of air was also demonstrated by determining the utilization of glucose. The procedure was the same as has been described. After 80 minutes of anaerobiosis the culture was opened and shaken in air. The Q-glucose for a culture, passed from air to nitrogen and passed again into air after growth was established, is shown in Fig. 7.

It is seen that the moment that the culture is without oxygen, the Q-glucose falls to 0 where it remains for 40 minutes and then rises abruptly to a value about 2.5 times the Q-glucose of aerobic culture. When the rate of glucose utilization in anaerobiosis is well established, passing the culture back into air produces a further increase in Q-glucose. This initial increase is followed by a rapid decrease until the Q-glucose is once again at the aerobic level. If D.N.P. is added at $5 \cdot 10^{-4}$ *M* at the time that the culture is passed into air, this decrease does not occur. D.N.P. was used at this concentration because it prevents growth but does not inhibit respiration.

The time where the Q-glucose has fallen to the aerobic value corresponds to the

References p. 573.

time where the fermentation has completely disappeared. Since the original increase in fermentation was at least ten fold, the disappearance is too fast to be accounted for by dilution due to the division of bacteria. Thus, the effect of D.N.P. appears to be due to inhibition of de-adaptation, not to the inhibition of growth. The increase in glucose utilization when an anaerobically growing culture is passed into air was found to vary from 5–30%. But, we have never observed a decrease in Q-glucose until after at least 30 minutes of aeration.

It has been found that there is no change in the cytochrome system in *E. coli* after growth in nitrogen[15]. As far as it is known the respiratory capacity remains unchanged. In the 30 minutes before de-adaptation starts, the glucose should be utilized by both the aerobic and anaerobic systems. In the ML strain of *E. coli*, under the best conditions, the glucose utilization during this critical period should approach the sum of the anaerobic Q-glucose and the aerobic Q-glucose, that is to say it should increase from, 1.7 to 1.7 + 0.7: an increase of 30%.

The two experiments reported in this section are not strictly comparable because of the differences in physiological conditions of the two suspensions. The suspension used for the Warburg studies, having been centrifuged, showed characteristically a low adaptation. However, with both suspensions the anaerobic fermentative capacity remained constant during at least the first 15 minutes of aeration. After this the fermentative capacity disappears very quickly. It is seen that D.N.P. inhibits this decrease in fermentation in the presence of air, as measured either by uptake of glucose or anaerobic production of CO_2.

DISCUSSION

The experimental evidence has been presented which indicates that in *E. coli* the increase in the fermentation in the absence of air is the result of the synthesis of an enzymatic system. A culture just transferred to anaerobiosis utilizes glucose at a negligible rate and is unable to grow. After 60–70 minutes the utilization of glucose has increased to a rate at least 2.5 times the rate under aerobic conditions and the growth occurs at very nearly the aerobic rate. This increase depends on the absence of air and requires a source of nitrogen. If air is re-admitted to the system the fermentative activity very quickly disappears. It also disappears in a very few minutes, even under anaerobic conditions, when the source of nitrogen is exhausted. The increase in fermentation, as well as its disappearance is prevented by 2,4-dinitrophenol. This is keeping with previous observations on the effects of D.N.P. on enzymatic adaptation[4,9]. The utilization of glucose under anaerobic conditions may also be inhibited by infection with bacteriophage just as is the utilization of certain adaptive substrates[5].

The adaptation as demonstrated in these experiments is remarkably labile, disappearing in air and in the absence of an exogenous nitrogen source. This does not invalidate the hypothesis that there is a synthesis of an enzyme or enzymes. Although there are adaptations which can occur in the absence of an external source of nitrogen, there are many which can occur only under conditions supporting growth (*cf.* [11]). Moreover, the "absence" of nitrogen is a relative condition and in many so called "resting" suspensions sufficient nitrogen may be released during the slight lysis which usually occurs to appreciably affect an adaptation.

SPIEGELMAN[12] and MONOD[11] have advanced the idea, considered under different forms by earlier workers[13] that the adaptive enzyme is stabilized by the presence of its

References p. 573.

substrate. This concept would offer an explanation of the rapid de-adaptation occuring as soon as air is re-admitted to a culture having a high fermentative activity. The degradative steps in the utilization of glucose by *E. coli* are not known. Some initial steps may be common to both the aerobic and anaerobic metabolisms. The two metabolic routes must diverge, however, at some point. It is reasonable to assume that, given conditions where the two pathways would be potentially functional, glucose would be preferentially metabolized through the aerobic system. When this is blocked, as in the absence of air, the last intermediate common to both systems would accumulate and provoke the synthesis of one or more strictly fermentative enzymes. The validity of the concept that metabolic intermediates initiate adaptations has been well demonstrated by the work of STANIER[14]. When air is re-admitted to the adapted system, the intermediate would again be removed by the aerobic metabolism and the adaptation would disappear.

This analysis of the system is also based on the conceptions of SPIEGELMAN[12]. He has postulated that the enzymatic constitution of a cell is the result of a competition between all enzyme-forming systems for the energy and protein material available for synthesis. The constitution at any given moment is determined by the synthetic capacity of the cell and the stabilizing capacities of the substrates present.

According to this theory, D.N.P. poises adaptive activity at any given level because it blocks the transfer of energy between different enzyme forming systems. The inhibition by D.N.P. of de-adaptation resulting from the absence of a source of nitrogen may be explained in this way.

These studies have been made on an organism where the respiratory activity does not change significantly after growth in the absence of air. The de-adaptation in the presence of air is probably adequately explained by the removal of the intermediate adaptive substrate. In other bacteria[15] and in yeast[10] however, where certain cytochrome components change or disappear during anaerobiosis, the fermentative activity would probably be even more labile due to the simultaneous re-adaptation of the respiratory system. The relative respiratory and fermentative capacity of a cell, at any given moment, may be interpreted as the result of the synthetic capacity of the cell and the effective degree of anaerobiosis.

Since the observations of PASTEUR[16] it has been known that the utilization of glucose by a living culture increases in anaerobiosis. He also showed that this augmentation is lost during growth in air. The present work has demonstrated that in *E. coli* this augmentation presents the characteristics of an enzymatic adaptation. It was also shown that when air is re-admitted to a culture after anaerobic growth there is a further increase in the glucose utilization due to the simultaneous activity of the respiratory and fermentative systems. After this initial increase the fermentative power is lost very rapidly.

One of the theories that has been advanced to explain the observations of PASTEUR (for review see[17]) is that air inhibits the function of some enzyme in the glycolytic cycle. If this were the case here, it is difficult to see how D.N.P. could inhibit both the increase and the decrease of the fermentative activity. The results suggest rather that air inhibits not the functioning but the synthesis of some strictly fermentative enzyme or enzymes.

ACKNOWLEDGEMENTS

The author appreciates her indebtedness to Dr ANDRÉ LWOFF for his friendly and generous interest and the privilege of working in his laboratory. She is also grateful to

References p. 573.

Drs. JACQUES MONOD AND MICHAEL DOUDOROFF for having suggested several critical experiments.

SUMMARY

Studies have been reported on the metabolism of a culture of *E. coli* passed from aerobiosis to anaerobiosis. During the first 30–40 minutes in the absence of air, there is no growth and no measurable uptake of glucose. The utilization of glucose starts abruptly at the moment that growth resumes and rises quickly to a maximum rate of about 2.5 times the aerobic rate. When air is readmitted to the system, the fermentative power disappears in a very short time. The fermentation also disappears within a few minutes in the absence of exogenous nitrogen, even under anaerobiosis. 2-4-dinitrophenol was found to stabilize the utilization of glucose at whatever rate it was at when the D.N.P. was added, preventing further increase or decrease in fermentative activity. These results have been interpreted as an adaptation of an enzymatic system for the anaerobic utilization of glucose. Their possible bearings on interpretations of the PASTEUR effect have been considered.

RÉSUMÉ

Nous avons étudié le métabolisme d'une culture de *E. coli* que nous avons fait passer d'aérobiose en anaérobiose. Pendant les premières 30–40 minutes en absence d'air, il n'y a pas de croissance ni de consommation de glucose mesurables. L'utilisation du glucose commence brusquement à l'instant où la croissance reprend et atteint rapidement une vitesse maximale qui est 2.5 fois plus élevée que la vitesse en régime aérobie. Lorsque l'on admet à nouveau de l'air au système, le pouvoir fermentatif disparait en très peu de temps. La fermentation s'arrête également en quelques minutes en l'absence d'azote exogène, même en anaérobiose. Nous avons trouvé que le 2.4-dinitrophénol stabilise l'utilisation du glucose à la vitesse où elle se trouve au moment de l'adjonction de ce réactif, empêchant ainsi des accroissements ou des diminutions de l'activité fermentative. Nous avons interprêté ces résultats en supposant une adaptation d'un système enzymatique à l'utilisation anaérobie du glucose. Nous avons pris en considération leur répercussions possibles sur des interprêtations de l'effet PASTEUR.

ZUSAMMENFASSUNG

Untersuchungen über den Metabolismus einer Kultur von *E. coli*, welche aus der Aerobiose in die Anaerobiose gebracht wurde, werden referiert. Während der ersten 30–40 Minuten unter Luftausschluss, wurde kein Wachstum und keine Glucose-Aufnahme beobachtet. Der Glucose-Verbrauch beginnt plötzlich in dem Augenblick, wo das Wachstum wieder einsetzt und steigt rasch bis zu einem Maximum, das 2.5mal so gross ist wie der Verbrauch in Anwesenheit von Luft. Wenn nun aufs Neue Luft zu dem System zugelassen wird, dann verschwindet die Fermentaktivität in sehr kurzer Zeit. Die Gärung hört auch in Abwesenheit von exogenem Stickstoff in einigen Minuten auf. Fügt man 2.4-Dinitrophenol zu, so wird der Glucose-Verbrauch bei dem Werte, den er im Augenblick der Zugabe besass, stabilisiert; weitere Zunahme oder Abnahme der Fermentaktivität wird so verhindert. Diese Ergebnisse wurden auf eine Adaptation eines Enzymsystems an den anaeroben Glucoseverbrauch zurückgeführt. Ihr möglicher Einfluss auf Auslegungen des PASTEUR-Effektes wurde erörtert.

BIBLIOGRAPHIE

1 J. MONOD, G. COHEN-BAZIRE ET M. COHN, *Biochim. Biophys. Acta*, 7 (1951) 587.
2 J. MONOD, *Ann. Rev. Microb.*, 3 (1949) 371.
3 J. MONOD, *Recherches sur la croissance des cultures bactériennes. Actualités scientifiques. Collection de microbiologie*, Hermann éd., Paris, 1942.
4 J. MONOD, *Ann. Inst. Pasteur*, 70 (1944) 381.
5 J. MONOD ET E. WOLLMAN, *Ann. Inst. Pasteur*, 73 (1947) 937.
6 S. S. COHEN, *Bact. Rev.*, 13 (1949) 1.
7 C. E. CLIFTON, *Advances in Enzymol.*, 6 (1946) 269.
8 R. J. WICZLER, *Science*, 99 (1944) 327.
9 S. SPIEGELMAN ET J. REINER, *J. Gen. Physiol.*, 31 (1947) 175.
10 B. EPHRUSSI ET P. SLONIMSKI, *Biochim. Biophys. Acta*, 6 (1950) 256.
11 J. MONOD, *Growth*, 11 (1947) 223.
12 S. SPIEGELMAN, *Cold Spring Harbor Symposia Quant. Biol.*, 11 (1946) 256.
13 J. YUDKIN, *Biol. Rev.*, 13 (1938) 93.
14 R. Y. STANIER, *J. Bact.*, 54 (1947) 339.
15 P. SCHAEFFER, private communication.
16 L. PASTEUR, *Etudes sur la bière. Oeuvres de Pasteur*, vol. 5, 1928, Masson éd. Paris.
17 F. LIPMANN, *Symp. on Respiratory Enzymes*, 1942, p. 48. Univ. of Wisconsin Press.

Reçu le 2 Juin 1951