# SCIENCE

VOL. 76 FRIDAY, DECEMBER 9, 1932 No. 1980

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SCIENCE: A Weekly Journal devoted to the Advancement of Science, edited by J. MCKEEN CATTELL and published every Friday by

THE SCIENCE PRESS

New York City: Grand Central Terminal
Lancaster, Pa. Garrison, N. Y.

Annual Subscription, $6.00 Single Copies, 15 Cts.

SCIENCE is the official organ of the American Association for the Advancement of Science. Information regarding membership in the Association may be secured from the office of the permanent secretary, in the Smithsonian Institution Building, Washington, D. C.

# MICROBIAL METABOLISM AND ITS BEARING ON THE CANCER PROBLEM[1]

By Dr. A. J. KLUYVER

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SOON after receiving the invitation to speak here to you on a biochemical subject I had the good fortune to come across Dr. Ellice McDonald's most illuminating paper published in SCIENCE last summer.

His condemnation of the trial and error method in cancer research and his convincing plea for a study of cancer on the basis of cell metabolism made it seem to me possible to speak to you on some results obtained in the metabolism of unicellular micro-organisms. The more so because investigations of the last decade tend to establish to a quite unexpected degree the unity in metabolism of all living organisms, either microbes or higher plants or animals.

It needs hardly to be emphasized that micro-organisms lend themselves quite exceptionally for studies in cell metabolism. Since many of them are readily available in pure culture, it is quite possible to start at any moment experiments with a uniform biocatalytic material. Moreover, we owe to the genius of Pasteur the remarkable observation that several of the colorless unicellular organisms can thrive in a medium which contains only one single organic compound besides the necessary mineral constituents. It is clear that this circumstance simplifies the study of metabolism quite especially.

We have profited by this favorable situation to study the metabolism of several microbes in somewhat more detail.

In considering the metabolism of any living cell one is immediately struck by the remarkable fact that the compounds in the food are never integrally con-

[1] Lecture before the Cancer Research Laboratories, University of Pennsylvania, Graduate School of Medicine, on May 2, 1932.

verted into cell constituents. On the contrary, a large part of the compounds of the food leaves the cell again after having undergone a frequently profound chemical alteration.

It is obvious that this part of metabolism, the so-called dissimilation process, is best accessible for closer investigation. In doing so we soon recognize that the dissimilation processes of various microbes can be divided into two types: either in a slow combustion of the food substrate with the aid of free oxygen, *i.e.,* respiration in the more restricted sense, or to chemical conversions of one or more constituents of the food in which free oxygen plays no part. The latter processes are those which are generally designated as fermentation and putrefaction processes.

Whilst the respiration processes proper do not offer to the chemically trained mind special difficulties for their understanding this can not be said of the fermentation processes. Here we are only too often puzzled by the diversity of the products formed, the constitution of which not seldom differs to a noticeable degree from that of the substrate. Moreover, the relative quantities of these different products may vary markedly under the influence of the external conditions, under which fermentation takes place.

In the best studied fermentation process, *i.e.,* the alcoholic fermentation of sugars by yeast, the situation is relatively simple, since about 95 per cent. of the fermented sugars can be recovered in two products—carbon dioxide and alcohol in equimolecular proportions. Here we may conclude that the chief event which has occurred is a conversion of the glucose according to the equation:

$$C_6H_{12}O_6 \rightarrow 2\ CO_2 + 2\ C_2H_5OH$$

But what shall we think of fermentation processes, such as those that are effected by butyl alcohol bacteria, by *Bacterium coli,* by *B. aerogenes,* etc., where we observe the glucose to be converted into numerous products occurring in variable proportions?

However, a closer investigation of all these very complicated processes has made it extremely probable that all these obscure processes are in reality nothing but a chain of consecutive primary reactions, all of which present a common character in so far that they prove to be reactions in which hydrogen is transferred from one molecule to another, or from one part of the molecule to another part.

Moreover, there are good grounds to conclude that the same holds good for the respiration process proper on the understanding that here we meet with a coupled dehydrogenation and hydrogenation in which free oxygen acts as a hydrogen acceptor.

Finally, there is some evidence that we meet with exactly the same situation in assimilation, *i.e.,* in those metabolic processes which lead from the food to the formation of cell constituents, a formation which of course is the first condition for proliferation.

Lack of time forbids me to enter here into a discussion of the arguments which have led to the establishment of this conception. I can only invite you to accept for a moment our general conclusion[2] that we may summarize the essence of biochemistry in the scheme:

$$\begin{aligned} &\text{I} && AH + B \rightarrow A + BH \\ &\text{II} && AH.B \rightarrow A.BH \\ &\text{III} && AH.B \rightarrow A + BH \\ &\text{IV} && AH + B \rightarrow A.BH \end{aligned}$$

It will be clear that if we accept this view it must have an important effect on our general outlook on metabolic processes, more especially on our insight as to the way in which biochemical conversions are brought about.

Since all primary reactions do not proceed in the absence of the cell, we must conclude that the cell contains agents which act as catalysts for the reactions involved. We owe to Dubrunfaut the first experimental proof that it is possible to isolate such catalytic agents—enzymes, as they are called nowadays—from the cells. Moreover, it has proved possible to make from the same specific cells preparations which are able to promote different reactions out of the many of which these cells are capable. This has led to the conclusion that a living cell should be considered as an arsenal filled up with enzymes which successively are brought into action.

Now it is clear that such a supposition would only be justified if every chemical reaction brought about by the cell required its own specific catalyst.

It is not possible to give here due consideration to the problem of enzymatic specificity. It may suffice to remark that this problem, which is generally discussed in relation to hydrolases, asks for a special treatment so far as the agents of oxidoreduction, to which the name of oxidoreductases can be given, are concerned.

With a view to the enormous number of substrates which are liable to dehydrogenation under the influence of one and the same specific cell, as was clearly demonstrated by den Dooren de Jong, the doctrine of extreme specificity of oxidoreductases becomes untenable. For it can scarcely be conceived that the cells of a bacterium contain as many dehydrases as there are suitable respiration substrates for these cells.

Moreover, since amongst the dehydrogenation sub-

[2] *Cf.* A. J. Kluyver, "Chemical Activities of Microorganisms," London, 1931.

strates we also meet with compounds which do not occur in nature, like bromopropionic acid we can not escape the conclusion that in this case the same catalyst is capable of acting upon different substrates and very probably even on a large number of these.

Once accepting the presence of such dehydrogenating "master-keys" in bacterial cells, there seems to be no serious objection to go farther on this way of simplification and to assume that in every living cell there is only a single oxydoreduction promoting agent, which is responsible for all oxidoreductions effected by the cell.

Although these conclusions have been reached as the result of a critical analysis of microbial metabolism, it seems extremely probable that they apply as well to the cells of higher plants and animals. If so, the chief contributions which the microbiologist has to offer to the human physiologist and pathologist is the insight that—apart from hydrolysis and its reversion —the whole of cell metabolism can be reduced to chains of coupled dehydrogenation and hydrogenation reactions and that in one cell there is only one specific catalytic agent which promotes all these reactions.

It is obvious that this view is not without bearing upon the cancer problem as well. For it would mean that the conversion of a normal tissue cell into a cancer cell could ultimately depend on a quantitative change in property of one single catalytic agent which determines metabolism.

The question arises: What do we know about this catalytic agent and more especially about the property which directs metabolism?

The answer can only be that the agent itself is practically inaccessible to direct investigation, since it can not be isolated out of the cell complex without seriously damaging its activity. However, it seems probable that we can learn something about the property in question by studying its influence on oxidoreduction systems present in the medium of the cells. This way seems the more promising, since we have in the determination of oxidation-reduction potential of the medium a convenient method for the measurement of the intensity of this influence.

In the course of time already numerous publications have appeared on the changes in oxidation-reduction potential in culture media as effected by the growth of micro-organisms. However, the greater part of these investigations is of an empirical character in so far as media of unknown composition have been used, and only exceptionally an attempt is made to trace the relations between metabolism and redox potential.

Still there is ample evidence of a more general nature that such a relation exists. Already the classical researches of W. Mansfield Clark leave no doubt that there is an intimate connection between the chemical activity of living cells in a medium and the potential of this medium. Later investigations from Coulter, Hewitt, Plotz and Geloso, Fildes and Knight, Lepper and Martin have fully corroborated this view. Nevertheless, in all these investigations the authors have not tried to relate the observed potentials with the chemistry of a special metabolic process.

A beginning in this direction was only made in a few papers. So Boyland brought experimental proof that the rate of cell-free alcoholic fermentation of sugar depended on the oxidation-reduction potential of the medium. Kusnetzow found the interesting fact that the direction in which glucose was converted under the influence of *Aspergillus niger* was materially affected by the potential or the pH of the medium.

The desirability of getting a better insight into the interrelation of metabolism and oxidation-reduction potential of the medium has induced my collaborator Elema[3] to further research.

From the start it was evident to us that it was of utmost importance to simplify the conditions of the experiments as far as possible. For this reason we have looked out for a metabolic process which can proceed in the absence of free oxygen, since the variable oxygen tension in different layers of culture media has often complicated the results of the earlier investigators. Furthermore, it was desirable that the cells involved would be able to show a normal metabolism in a simple synthetic medium.

We have found these conditions fulfilled in making use of denitrifying bacteria, of which several species are able to thrive quite vigorously in a simple medium prepared from tapwater with the addition of ethyl alcohol, potassium nitrate and some phosphate.

As a first result may be mentioned that also in this synthetic medium reproducible changes in potential occurred which were highly independent of the nature of the electrodes used, but which were evidently determined by the metabolic processes effected by the bacteria, since in the absence of bacteria no specific potential could be observed. This may be deemed remarkable, since in this case the cooperation of other oxidoreduction systems than those which originate from the bacteria in the establishment of the measured potential difference is excluded.

In studying the changes in potential during the course of the development of denitrifying bacteria, we were soon struck by the fact that after an initial period, in which evidently the electrode was not yet

[3] *Cf.* B. Elema, "De bepaling van de oxydatie-reductie potentiaal in bacterien cultures en hare beteekenis voor de stofwisseling," Delft, 1932.

adjusted, the changes were dominated by simultaneously occurring changes in pH, to the understanding that there was an unmistakable parallelism between the curve of the redox potential and that of pH. However, finally always a sharp drop in potential occurred which was not accompanied by a corresponding drop in pH.

Our attention was drawn to the remarkable circumstance that this final drop in potential coincided with the disappearance from the medium of an intermediately occurring metabolic product—the nitrite.

So here—as far as is known for the first time—a direct relation between the oxidation-reduction potential and a definite metabolic product was traced.

This view was fully confirmed by further investigations in which the denitrification of nitrite itself was studied. In these experiments the necessity of growth of the bacteria was excluded by raising the number of the cells in the suspensions. Under these conditions it could be ascertained that at any moment—as long as nitrite was still present—the potential observed was directly dependent on the pH, at least as long as a value of 9.1 had not yet been attained. With a further increase in pH the potential remained constant at a definite level until the nitrite again had been consumed.

These results were completed by the observation that in a medium from which the nitrite had disappeared, the original value of the potential was immediately restored by the addition of the smallest trace of nitrite. However, the concentration of the nitrite proved to be without any influence on the potential.

If we try to interpret these findings we must conclude that from the several reversible oxidoreductions which proceed in rapid succession at the surface of the catalyst, the reaction with the slowest rate, *i.e.*, the reduction of the nitrite, is determining the oxidation-reduction potential in the medium. But since the concentration of the nitrite in the medium is of no importance we may infer that only the small part of the nitrite that is adsorbed at the catalyst participates in the oxidoreduction system that primarily is responsible for the potential measured.

This is a further proof that this potential is a characteristic result of the interaction between metabolic substrate and the heterogenous catalyst.

With this insight gained it seemed of importance to investigate in how far the measured potential could be influenced by adding to the medium typical reversible oxidoreduction dyes like methylene blue, etc. *A priori* it seemed quite possible that addition of minute quantities of these dyes would have no other influence than an acceleration of the adjustment of the electrode to the surrounding medium.

In this line of thought several investigators have added very small quantities of oxidoreduction indicators, tacitly assuming that this addition would not alter the oxidoreduction state of the medium under investigation. Whilst this is true in those cases wherein true equilibria of sufficient capacity are studied, our experiments have shown that this does not hold good in the case of stationary reaction as occur under the conditions chosen by us.

On the contrary, the experiments made left no doubt that even traces of oxidoreduction indicators markedly influence the redox potential of the medium. We are inclined to attach rather great importance to this result with a view to the intimate connection which evidently exists between metabolism and redox potential in the medium. If we are entitled to refer this potential under normal conditions to the metabolic processes of the cells which are present in the medium, then we must conclude that changes in this potential mean a change in metabolism of these cells. We might look upon it in this manner that the stationary reaction state is modified by the interference of the added indicator, as a hydrogen donator in its reduced form as a hydrogen acceptor in its oxidized form. This will imply that the normal metabolic oxidoreductions will be changed at least in a quantitative sense, but it is also quite conceivable that the change in potential which results from the addition of the oxidoreduction system will prevent the occurrence of special oxidoreduction reactions of the cells or promote others.

If, in concluding, I may venture to dwell for a moment on the possible significance of these observations for the cancer problem, I should like to make the following remarks.

The cornerstone of our knowledge of the metabolism of cancer cells is nowadays still—in spite of some incident adverse criticism—the fundamental discovery made by Otto Warburg of the prevalence of glycolytic activity also under aerobic conditions.

Although nothing can be said with certainty, this makes it probable that metabolism in a cancer cell will induce a more reduced level, *i.e.*, a lower reduction potential than the normal cell will have, owing to its metabolism in which free oxygen takes a preponderant place.[4]

This hypothesis is materially supported by the important findings of Albert Fischer, of Copenhagen, on the increased proteolytic activities of pure cultures of cancer cells as compared with normal ones.

[4] This opinion does not necessarily conflict with the recent observations of Waterman, who reports that serum of cancer tissue had a higher potential than that of normal tissue, since these measurements relate to media in which the living metabolizing cells were absent. *Cf.* Acta Brevia Neerlandica 1, p. 188, 1931.

For the investigations of Grassmann and of Waldschmidt-Leitz have shown beyond doubt that proteolysis is a process which is strongly intensified by natural activators like glutathione, but only in so far as they occur in the reduced state. The strict correlation of reduced state and proteolysis is moreover clearly demonstrated by the general occurrence of low potentials in cell suspensions as soon as active metabolism ceases and autolysis sets in.

In this connection it is tempting to see a cancer cell as a cell in which autolytic processes have overcome the opposing forces of metabolism, but which on their way to complete autolysis have found a new stationary state at the moment of the passing of the potential which corresponds to glycolysis.

At first sight one might therefore expect that it should be possible to restore the respiratory function of the cancer cell by adding to the medium a reversible oxidoreduction system by which the potential of the environment and therewith the potential at the catalyst would be raised. The mentioned effect of the addition of oxidoreduction system on suspensions of denitrifying bacteria proves the appropriateness of such a procedure.

Moreover, the discovery of Barron and Harrap of the methylene blue respiration of erythrocytes gives direct proof that it is possible to change by this means a glycolytic metabolism into a respiratory one.

However, there is sufficient evidence in the work of Warburg, Kisch and others that the same step with cancer cells is ineffective.

It seems to me that this negative result is of far-reaching significance. For we must conclude that the change in metabolism which a normal cell undergoes by its transformation into a cancer cell does not have its primary cause in the lowering of the potential at the catalyst with the reduction of respiration as a consequence. On the contrary, all points to the probability that inversely the downfall of the potential is caused by the decline of the respiratory function.

This must be deemed quite possible on the basis of the duality of the given theory of respiration. In contrast to fermentation, respiration asks for a special oxygen activating apparatus besides the biocatalyst which primarily is responsible for the oxidation-reduction potential in the cell and its environment. A defect in this oxygen activating apparatus—Warburg's "Atmungsferment"—will suffice to diminish or prevent normal respiratory activities. The result will be a lowering of the potential which ultimately will lead to autolysis and death, but which, under special conditions, may find a new stationary reaction state at the basis of a glycolytic metabolism. In this line of thought there does not seem to be much hope for the restoration of a cancer cell into a normal one.

It may be remarked, by the way, that this representation admirably fits in with the hypothesis formulated quite recently by Crawley in his recent paper,[5] according to which the essential difference of malignant cells from the normal ones is to be found in the fact that the protein sols of the former are in a chronic state of excessive peptization, *i.e.*, the micelles are smaller than those occurring in normal cells.

Still it would be premature to conclude from the foregoing that cell metabolism in its present state of development has nothing to offer to cancer research. For, although attempts to bring the cancer cell back to its normal state may not seem promising, still it might *be possible to prevent its proliferation.*

Indeed, there seem to be several indications that we have in the maintenance of relatively high potentials a decisive means of preventing those hydrogenations which are essential for the formation of new cell substances, *i.e.*, for growth of the cells.

The first time that my attention was drawn to this point was on occasion of some observations made by Visser 't Hooft in connection with the metabolism of acetic acid bacteria. Visser 't Hooft found that, by increasing the rate of aeration of media inoculated with an acetic acid bacterium, rather soon a situation was reached in which the growth of these bacteria was altogether stopped. Nevertheless, a preformed population of these bacteria were perfectly capable of oxidizing different respiration substrates by aeration intensities which surpassed markedly the critical value for growth.

But also the denitrifying bacteria show a quite similar behavior. From the graphs in Ellma's dissertation, it may be derived that by inoculating denitrifying bacteria in an alcohol-nitrate medium in the phase of active growth, potentials are attained which are far lower than those which result afterwards from the intermediary production of nitrite. There are good reasons to conclude that as soon as the higher "nitrite potential" is established growth has altogether stopped. This would explain the rather well-known but hitherto poorly understood phenomenon that in an alcohol-nitrite medium inoculations with denitrifying bacteria are unsuccessful, notwithstanding—as we have seen before—denitrification proceeds vigorously if a preformed mass of these bacteria are added to the same medium. Obviously, the potential maintained by the presence of nitrite is too high to allow of assimilatory dehydrogenations.

It may be deemed probable that the same will hold good for the cancer cell and that *its proliferation* will be checked by maintaining in its medium a potential which will be too high for the dehydrogenation reactions which are essential for growth. Moreover, it

[5] *Jour. of Phys. Chem.*, 36: 1282, 1932.

does not seem excluded that by adding suitable oxido-reduction systems the critical growth potential can be surpassed to such a slight extent only that the critical growth potential of normal cells is not attained.

It will be clear that thoughts like these do not pretend to be more than mere suggestions for future investigation. But of one thing I feel quite sure, *i.e.*, that the solution of the cancer problem will be the result of a deeper insight into the phenomena of cell metabolism, an insight that will be gained only by further investigation of pure cultures either of cancer cells themselves or of unicellular organisms.

# HOW THE PRIMITIVE ANTS OF AUSTRALIA START THEIR COLONIES[1]

By Professor W. M. WHEELER

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THE great naturalist and physicist, Réaumur, was the first to attempt an investigation of the founding of colonies among ants, in 1743. Since that time the problem has become more complicated and we now know that these insects practice at least four different methods of colony-founding. Three of these have been satisfactorily elucidated within the past thirty years. A year ago I was able to detect a fourth, quite unexpected method in several Australian species of the very archaic and primitive subfamily Ponerinæ. All four methods represent so many peculiarities in the behavior pattern of the young, recently fecundated female or queen, and these in turn depend on the amount of her food-reserves in the form of adipose tissue and wing-musculature. Species in which these reserves are deficient are compelled to adopt one of two *dependent* methods of colony formation: the young fecundated queen either leaves the maternal colony accompanied by a band of workers that assist her in establishing a new nest and community or she becomes parasitic in a colony of an alien species. The former method is a kind of swarming analogous to that of the honey-bee, but differs in being practiced by the young queens instead of by the old mother queen of the colony. The parasitic ants are of unusual interest, but I shall consider only the two remaining methods, which are of the *independent* type.

Nearly all our ants belong to the four most recent and most specialized taxonomic subfamilies (Myrmicinæ, Pseudomyrminæ, Dolichoderinæ and Formicinæ) and found their colonies in the following manner: After fecundation by the male during her marriage flight, the large-bodied queen, which has been plentifully supplied with abdominal fat during her larval life, descends to the earth, discards her wings and immures herself completely in a small cell in the soil, under a stone or under the bark of a log. The voluminous wing musculature in her thorax, now useless for purposes of flight, at once begins to break down and dissolve in the blood, so that its proteins, together with the fat in the abdomen, can be used as food by her developing ovaries. These food-reserves enable her not only to mature a number of eggs but also to rear some of the resulting larvæ as a small initial brood of diminutive workers. During this period, which may extend to eight months or even more than a year, she manages to live exclusively on her own tissue-reserves and to feed her larvae with saliva. She also normally devours many of her own eggs and larvae or feeds them to their sister larvae. She often concentrates her care on one or a few of her offspring, so that these pupate and emerge as minute workers before the others. As soon as they are thoroughly mature they break out of the cell and secure food for themselves, their famished mother and the still undeveloped portion of the first brood. They now take over the control of the colony, provide all the food for the diminutive community and thus enable the queen to specialize henceforth as a mere egg-laying machine that supplies them with successive broods to rear. They begin to expand the nest by excavating additional cells and galleries and the colony grows apace, till its trophic status is so favorable that after a few years it can produce males and young queens. This method of colony formation I have called the *perfectly claustral method.*

I find that the Ponerinae present a very significant variant of this form of colony-founding behavior. More than thirty years ago I showed that these ants do not feed their larvae with liquids by regurgitation, but in a much more primitive fashion, with pieces of freshly killed insects. In all the intervening years I have sought their method of colony-founding, but in vain, and other myrmecologists have had no better success. While accompanying the Harvard expedition to Australia, which has the most superb Ponerine fauna of any continent, I succeeded in filling this gap in our knowledge. The richness of the Australian Ponerine fauna is shown by the fact that at least 300, or 25 per cent., of the 1,200 species, subspecies and varieties of ants now known from the island con-

[1] Read at the meeting of the National Academy of Sciences, University of Michigan, on November 15, 1932.