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MICROBIAL METABOLISM AND AGRICULTURE[1]

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THIS paper is designed to point out certain relationships between fundamental studies on microbial metabolism and the great basic science and art of agriculture, and that these advances in physiology, enzymology and physiological chemistry are significant not only in terms of human nutrition and human physiology and curative and preventive medicine, but also in farm production.

Obviously I am not called upon on an occasion of this kind to explore to the limits all the ramose diverticula of modern findings and theories relative to the uptake, synthesis and breakdown of substances useful or harmful to the microbial cell. Rather I should indicate those studies and advances which seem particularly promising in their application to non-microbial organisms. There is a great practical significance to the present and mounting knowledge on microbial metabolism.

Studies on microbial metabolism have stimulated a greater appreciation of the real unity of the basic characteristics of all protoplasm and the wide-spread uniformity of many of its activities. First an example: Until comparatively recently the ability of plants to fix carbon dioxide was conceived to be quite dependent upon the presence of chlorophyll. Then Winogradsky and others found that certain microbes were able to fix carbon dioxide without chlorophyll, and so living things became segregated into those which could not use carbon dioxide and those which could—the latter being subdivided into those which required chlorophyll and light (the photosynthetic forms) and those which utilized reduced inorganic

[1] Address of the retiring vice-president of Section O—Agriculture, American Association for the Advancement of Science, Cleveland, Ohio, September 14, 1944.

compounds such as hydrogen sulfide or ammonia as hydrogen donors in the reduction of the carbon dioxide. Then came the brilliant work of Gaffron, van Niel and others, which revealed that some kinds of organic compounds may replace inorganic when used by certain so-called chemosynthetic bacteria. And then it was found that the exact period during which a green plant was illuminated did not necessarily precisely coincide with the period during which carbon dioxide was being fixed. And then the pioneer work of Werkman and Wood and their colleagues showed that certain bacteria, in the first instance the propionic acid bacteria, can and do fix carbon dioxide, even though they are included in the heterotrophic group. This was revolutionary thinking, quite against the traditions of the texts. But these men by use of the isotopic techniques not only confirmed their earlier findings, but showed the channel through which in this case the carbon dioxide enters into and becomes a part of an organic molecule. Then it seemed that we might recognize three groups of carbon dioxide-fixing organisms, the photosynthetic, the chemosynthetic, certain heterotrophs. But what about the animal kingdom which is entirely heterotrophic? Soon came the evidence that animal tissues likewise may assimilate carbon dioxide, even the tissues of the human body. Through this sequence of studies we have a most surprising and emphatic demonstration of wide-spread uniformities in metabolism of plants and animals, of the essential unity of protoplasm. Studies on bacterial metabolism are bringing us to an understanding of how the corn plant manufactures starch. True, one channel or method only whereby carbon dioxide gets into an organic molecule has been demonstrated for certain bacteria. But it seems improbable that the one first discovered is either the only or necessarily the most important channel or mechanism of carbon dioxide fixation by plants. When we look back upon the development of our knowledge in this field we realize that a part of our difficulty has been slowness to recognize the applicability of certain principles of physical chemistry, such as the reversibility of chemical reactions. May I again emphasize that studies in bacterial metabolism, studies on forms of life possessing no photosynthetic powers, are giving answers to phenomena which are fundamentally basic to agriculture. The apparent ease and facility with which the agricultural and forestry crops manufacture carbohydrates from carbon dioxide and water has irritated and baffled the plant physiologist and the agricultural scientist. Apparently studies on microbial metabolism are furnishing needed keys and tools.

We are woefully deficient in our knowledge of certain phases of nitrogen metabolism of living cells. One of the most puzzling problems since the beginnings of modern chemistry has been the fact that although nitrogen is an essential element in all living protoplasma, and though elementary nitrogen is almost omnipresent, relatively few cells seem able to bring about its fixation and utilization. Nitrogen fixation is one of the really fundamental problems of agriculture. We know that certain free-living soil bacteria and algae can fix nitrogen and build it into protoplasm. We know too that certain bacteria and perhaps other micro-organisms in symbiosis with higher plants can also fix nitrogen. Upon these two facts, so far as our present knowledge goes, we base our whole theory of native soil fertility. The Haber process of nitrogen fixation and its later developments have conceivably made possible an agriculture freed from a base of microbial nitrogen fixation. But far more nitrogen is fixed microbially now, and probably will continue to be, than will be fixed by artificial means. One hesitates to predict, but this situation will probably continue until we have learned just how the microbe fixes the nitrogen. Progress is being made. The work of Virtanen and of Wilson and their collaborators has yielded and is yielding results. It is probable that presently the technique of fixation and the nature of the initial compound or compounds will be clear. What this may mean for practical agriculture is not entirely certain. But we shall know a mechanism which has long baffled us and which may clear up the long-term enigma of the nitrogen-fixing powers of the *Leguminosae* and of some other groups of higher plants.

The chemical changes which micro-organisms bring about in their environment are of real significance in agriculture as well as in other fields. Our knowledge of the steps which are required and of the tools which are used by the organism is gradually being unraveled. We live in a welter of cycles named after this and the other individual; chains of chemical transformations which have been suggested as hypotheses. And each comes nearer to giving us a clear picture of what the cell is doing, what tools it uses and from whence the energy is derived to keep the machine functioning.

The microbial cell has many advantages in the study of certain physiological processes over the cells of multicellular forms. One may have uniform cells, cells at various growth stages, with or without chlorophyll, and with an astonishing gamut of uniformities with which to work. The extension to study and confirmation in physiology of higher plants is proving most useful in an understanding both of normal and abnormal cell physiology.

Micro-organisms have long been used as food, but their production as crop plants has only recently been initiated and somewhat developed. The most outstanding and consistent of the physiological differences between animals and plants in general is the lack of ability on the part of animals to utilize nitrogen in a form simpler than an amino acid, or at least an amine. Most plants on the other hand can utilize ammonia and even nitrates and other simple nitrogen compounds. From the standpoint of agriculture, in most of our agricultural economies, there is an overbalance of carbohydrate feeds as contrasted with protein, particularly in production of meat, milk and eggs. It is true we may apply the nitrogen to the soil and get crop increases, but with still some inbalance. In recent years there has come the thought that it might be practicable to convert the excess carbohydrates, lignins, etc., into proteins through the intermediation of micro-organisms. Several examples of this tendency may be cited. Previous to the outbreak of this war, a considerable amount of German literature appeared on the subject of developing supplementary protein feeds from cheap carbohydrates and synthetic nitrogen. The fact was established that the yeast cells contained in brewers-grains, distillery slops, etc., make a distinct contribution to the protein value of these as feeds for domestic animals and birds. By shift of emphasis, cheap carbohydrates were sought on which to grow yeasts primarily for their nutritive value. Wood waste, sawdust, was hydrolyzed, the syrup extracted, synthetic nitrogen (ammonia) added and yeasts grown under appropriate conditions of aeration. A reasonably complete conversion of the nitrogen into protein useful for feeding hogs, poultry and other domestic animals resulted. This, of course, in the interest of a self-sufficient economy.

More recently we have been told of the possibility of utilizing the yeast as a human food, not simply as a supplier of certain vitamins, but, as the newspapers had it, in the form of synthetic beef steaks. Work of the Lindegrens in hybridizing the yeasts to get better varieties may well be significant.

But yeasts constitute a tiny fraction of the range of micro-organisms and higher fungi that can attack carbohydrates, even lignins, and which can utilize ammonia with consequent production of proteins. Some development is possible in our post-war world in the utilization of these materials together with our excess production of synthetic nitrogen in the manufacture of cattle and hog feed. Xylans are available in abundance; the production of xylose syrups is feasible. Micro-organisms and fungi are known which can utilize one or the other or both. Great quantities of coarse fodders, cull potatoes, etc., might with some clever planning be converted into high protein feeds, into protein of good quality. We know comparatively little about the fundamental metabolism of most micro-organisms and fungi. But there is little doubt but what a fungus agriculture might put to good use a great amount of present waste and cull material.

Recently we have become increasingly aware that the microbial flora already present or which may be developed in the body of certain animals may be distinctly useful. We now know that urea can at least in part replace proteins when fed to certain ruminants, including the dairy cow. Apparently there exists in the paunch of these animals a microbial flora which utilizes on the one hand the carbohydrates of the food but metabolizes also the urea into the protein of its own cells. Probably the organisms are bacteria. But just how the bacterial protein is utilized by the animal we are not quite sure. Much work is needed by a combination of bacteriologists, protozoologists and physiologists. We know that not only does the paunch contain a rich flora of bacteria but an unusual and startling fauna of protozoa. The recent success of Hungate[2] in cultivating a *Eudiplodinium* from the paunch of cattle is a challenge to further work. These obligately anaerobic ciliates were cultured for 22 months in a medium containing inorganic salts and pulverized dried grass and cellulose. This author shows that not only does this protozoan digest cellulose and contain the enzyme cellulase, but that the synthesis by protozoa of proteins in the paunch supplies perhaps one fifth of the protein requirements of the animal. Here we have an intricate symbiotic mixture of micro-organisms, protozoa feeding on bacteria and cellulose; and the cow utilizing both as protein food. Here certainly we will find eventually close relationships between microbial metabolism and agriculture.

Brilliant work is being done on the characterization of foods and feeds on the basis of the adequacy of their proteins, their amino-acid makeup and their association with vitamins and accessory growth substances. Agriculture benefits from both sides: it produces the food, and it uses the feeds. Analyses for amino-acid content and particularly for vitamins and other accessory growth substances are increasingly important. Here again microbial metabolism is playing an important role. Many of the bacteria are able to synthesize all the various growth accessory substances which they require. Some produce vitamins to such a degree as to be significant when used as foods or feeds. but many others are "born" deficient in ability to produce certain of these. This is particularly true of species that have become highly adapted to a particular environment. Such are to be found among the bacteria parasitic on plants and animals, and others which grow normally on fermenting plant materials. The environment has supplied their need for auxins or vitamins, etc. We find them difficult in consequence to culture, particularly on a synthetic medium. It was not at first clearly recognized that certain of these growth accelerants, etc., required by these organisms were identical with the vitamins. And now we find that these organisms are becoming most useful

[2] *Biol. Bull.*, 83(3): 303–319, 1942.

tools in evaluating the vitamin content of foods. And the same is true for the amino acids. Some organisms can synthesize from the simplest materials all the amino acids which they need. Others can not. There is good evidence that such organisms lack some essential part of the enzyme system necessary for the synthesis of some particular amino acid or even several amino acids. Unless these are supplied ready manufactured, the cell can not grow. And, other conditions being favorable and standard, the rate of increase in the microbial cells is an easily ascertainable mathematical function of the amino-acid content of the medium. The rate of growth becomes a tool for determining quantitatively the amino acid.

Our armamentarium for this purpose has been enriched from two principal sources, the isolation of organisms from special environments and the development of mutations. The latter has been most extensively studied and developed by Beadle for the fungus *Neurospora*. Various mutations have been developed which reveal certain enzyme deficiencies. In some cases fruiting is inhibited by lack of certain substances in the medium. It is evident that those scientific laboratories which service agriculture have now a new array of tools; biology has come to the aid of chemistry in much the same fashion as in the past chemistry has tooled biology. Facility in determination of the quality of foods and feeds will have its abundant fruition in better nutrition for man and animal.

For decades there has been accumulating a considerable microbial literature on the general topic of antibiosis and of staling substances. Observation has long shown that certain types of organisms grow better together, but in other cases one organism may be antagonistic to another. It is common observation that when growing upon a culture medium mold hyphae show either positive or negative chemotropisms. A microscopic view of the margin of certain mold colonies shows that the branches of the filaments as they originate quite accurately bisect the angle between the adjacent hyphae. Evidently the young hyphae grow in the direction of the lowest concentration of excretory products, or conversely show a negative chemotropism with reference to the excretory products of the plant itself. When two colonies approach each other in growth, they are mutually inhibited and do not grow together; *i.e.*, they remain separated. Then, too, one species may inhibit the growth of another. The medium on which an organism has been grown may be positively inhibitory for another. The pyocyanase produced by *Pseudomonas aeruginosa*, actively lytic for certain other bacteria, has long been proposed as a therapeutic agent. Recently major attention has been drawn to these staling or antibiotic or bacteriostatic or bacteriolytic substances when it has been found that some were far more toxic or inhibitive to certain pathogenic organisms than they are to the tissues of the animal body, and we had the discovery and development of penicillin and the various other microbial bacteriostatic or bacteriotoxic substances. And more recently we are gaining an insight into what essential segments of the enzyme systems are put out of commission. We have been so impressed by the efficiency of penicillin in human therapy that we may overlook the agricultural significance of future developments of this type in study on microbial metabolism. The utilization of therapeutic agents in veterinary medicine will follow the regular course of human medicine. But there is a wide-open field in the control of plant diseases, particularly seedling diseases, damping-off, etc. We in agriculture are coming to a realization that fungi attacking roots and seedlings play a great role in limiting crop production. Certain of the pythiaceous fungi are particularly significant. When grown on culture media, inhibition by various fungi is readily observed. A whole field of phytic therapeutics is opening up. The antagonisms and interactions of the fungi and the bacteria make possible the production, isolation and identification of antibiotics, some of which may be useful, and at least will give us clues to work with in our efforts to develop synthetic plant treatments and to explore more adequately the relationships of chemical structure to therapeutic efficiency and toxicity.

Another type of advance which we in agriculture view with great interest is the progress that is being made in determination of the paths of microbial synthesis. I remember years ago—now nearly four decades ago—hearing Dr. Nef, then head of chemistry at the University of Chicago, predict that largely as a result of the analytic studies underway, adequate techniques of synthesis of the carbohydrates would be commercially feasible within a decade. But it has not yet been accomplished. The problem of synthesis is far more complex than originally surmised. But we are making progress. By use of enzyme preparations we are able to carry on these syntheses apart from the living cell. Witness the recent success of Barker and his collaborators in making use of an invertase-free sucrose-phosphorylase from the bacterium *Pseudomonas saccharophila* in the synthesis of sucrose from glucose-1-phosphate and fructose. We are beginning to see how old Mother Nature is able with little apparent effort to synthesize the complex compounds which she produces so abundantly in every green plant. And our microbial studies are increasingly significant.

Knowledge of microbial metabolism is bound eventually to crack the great mystery of virulence in disease production. We know rather accurately what changes certain bacteria produce upon their environment *in vitro,* but our efforts to correlate with what happens *in vivo* have not been particularly successful. One of our difficulties has been that in most cases we could not readily determine degrees of virulence as contrasted with degree of resistance or immunity. Recently it seems that we are on the track of techniques which should help in unraveling the difficulty. We have learned that measurement of virulence of an organism by the determination of the size of the lethal dose is not entirely satisfactory. Some studies underway on maize show one advantageous technique. Inbred strains of maize become highly homozygous, and the individuals of each strain react with a high degree of uniformity. And by choice of these inbreds one may secure a regular gamut of resistance to certain diseases. Particularly interesting is that caused by *Pseudomonas stewartii,* the Stewart's disease of corn. Some strains of corn are highly resistant to infection, others very susceptible. Furthermore, the causal organism, Stewart's bacterium, can be caused to mutate, and one may secure strains varying widely in attacking power of virulence. We have fixed standards to measure virulence; we have strains which mutate when grown in culture media. But we need vastly more knowledge of the slight metabolic differences among these microbial strains. Variations in virulence presumably must have a basis and be completely correlated with variations in the microbial metabolism. We are gaining command of the tools which are needed to detect these changes. When once we know the metabolic differences among these strains, we shall be for the first time in position to know what constitutes immunity. In this whole series of studies, agriculture will have much of profit.

Agriculture has tremendous interest in the changes which may be effected by micro-organisms in their chemical environment. The studies on basic microbial metabolism are yielding the keys to understanding of the exact chain or sequence of events in the great maze of fermentations and products of microbial change. Higher plants produce vast stores of polymers based upon the hexoses, and upon these most of our present fermentation industries are based. But plants also produce vast stores of polymers based upon the pentoses as well. Pentose sugars could easily be made available in large amounts if we knew satisfactory fermentative uses for them. The sugar xylose is readily secured from many agricultural products. It is not utilized by our domestic animals. It can be attacked by certain micro-organisms. But we need more and better basic information on microbial metabolism before we can satisfactorily unlock effective utilization.

Let's look for a moment at the industries and potentialities based largely upon the use of hexose sugar.

Alcoholic fermentation has long been studied intensively. Pasteur showed the relationship of the yeast to the process; Harden started our modern development of knowledge relative to the enzyme systems in all their complexities. While there is much still to be done, it probably is safe to say that more is known relative to the changes from sucrose or starch to alcohol and carbon dioxide than to those inherent in any other metabolic series. In many respects the studies on this sequence of changes have been basic to our understanding of many other intracellular types of changes. In these studies agriculture has a tremendous stake.

Before exploring this phase of fermentation further, let's look for a minute at the sources from which we may secure the carbon for the multitudinous organic compounds with which civilized man is increasingly concerned. We live in an ocean of air-containing carbon dioxide, and we have access to the oxidized carbon given off in flue gases, and to that found in the great deposits of carbonates in the earth. Carbon dioxide itself is, of course, increasingly useful to man as such. But carbon in most cases is in reduced carbon compounds in which there are carbon-to-carbon linkages, or in which there is hydrogen or hydroyxl, etc. Technology has methods of reducing the carbon of carbon dioxide, but for all practical purposes the organic compounds of modern life have their origin in the growth of plants. The marvelous precision with which plant life takes up carbon dioxide and reduces it and combines it has proved thus far to be our cheapest and most satisfactory technique. True, we may start with coal or with petroleum, but these are but the fossilized products of plant growth. A major economic concern of agriculture is just this: What are the relative efficiencies in securing the organic compounds needed by civilization from coal, from petroleum and from the products of farm and forest? There are broad areas in which each has an advantage. In petroleum and in coal there are already present many useful compounds which only require extraction; many of the products of agriculture, particularly for use as food and feed, have not been and probably can not be successfully duplicated. But there are considerable areas where relative advantages of crops, coal and petroleum are not so clear. For example, one may produce alcohol synthetically from coal, from petroleum, from agricultural products or by fermentation from agricultural products. Concretely at present it breaks down into competition between fermentation alcohol or synthetic alcohol from coal and petroleum. For some decades organic chemistry was coal tar chemistry and ring compound chemistry, and more recently it has been petroleum chemistry and straight chain chemistry. You will recall the ballyhoo two years ago about how our synthetic rubber problems would be promptly settled by petroleum. The paper chemistry of butane or ethylene to butadiene to rubber is beautiful to behold. But American tanks and guns and jeeps are in action all over the world because yeasts made alcohol from sugar and chemists made this fermentation alcohol into butadiene and this into synthetic rubber. It is true that in this great crisis in motorization and mechanization it was the zymotechnologist and not the petroleum chemist that made rubber supplies possible and reasonably adequate. May I repeat my point. There are many areas in which needed organic compounds can be produced either by fermentation, *i.e.*, by microbial activity or by chemical synthesis. The calm assumption of some of our chemist friends that huge retorts and distillation devices and catalysts and high pressure, etc., constitute the answer should be regarded in many cases with some scepticism. Economics must determine what process is most efficient. The fact that quinine has been synthesized at Harvard recently doesn't mean that the Cinchona tree may not be the most satisfactory source of the drug any more than the demonstration of an enzyme system that can combine fructose and glucose into sucrose means that we will quit producing sucrose from the sugar cane in Cuba. I am insisting that a practical chemical economy in the future depends quite as much upon our exploration of the fundamentals of microbial metabolism as upon the exploration of petroleum chemistry, conceding the vast importance of the latter. A substantial part of the economics of agriculture, of the future of agriculture, is bound up in the adequate exploration of microbial metabolism.

Fermentation products frequently have an advantage in that they are relatively pure, or may be readily purified. Commonly they are not mixed with isomers. For example, 2-3 butylene glycol is a product which can be produced commercially by fermentation with facility. But there are several isomers of this compound which show interesting difference in behavior. One apparently would make a good anti-freeze for your automobile radiator, the others will not. Chemical synthesis is apt to yield a mixture with great difficulty in separation. But certain bacteria produce the desired isomers practically pure.

The stake of agriculture in the problems of chemical utilization are considerable and material. Right now millions of bushels of grain are being converted into rubber. To what extent may agriculture look to fermentation industries in the future to assist in relieving us of great surpluses of grains, surpluses which are absolutely inevitable without major changes in world economy, changes which at the best can be brought about but slowly? Crop residues likewise must be studied directly as competitors of coal and petroleum in the future development of our agricultural economy. Fermentation products will increasingly constitute the raw materials for chemical synthesis and transformations.

In summary, microbial metabolism, microbial nutrition, real continuance of research in fundamental fields relative to the physiology of micro-organisms, are of quite as much significance to agriculture as to medicine or to biology as a whole. We are on the verge of new advances which may have the most far-reaching consequences to standards of living and to civilization.

OBITUARY

ROBERT BENNETT BEAN
1874–1944

ONE of America's outstanding anthropologists and teachers of anatomy, Dr. R. Bennett Bean, died on August 27, 1944, in Staunton, Virginia. He had resigned from his position as head of the department of anatomy in the University of Virginia School of Medicine two years previously because of ill health brought on by arteriosclerosis. His death was not unexpected but was a source of grief to many friends, associates and former students as well as his family.

Dr. Bean was born at "Pleasant Hill," Gala, Botetourt County, Virginia, on March 24, 1874, the third of a family of eight children born to William Bennett and Arrianna Williamson Carper Bean, whose ancestors had been early colonists of Virginia and Maryland, respectively. His father had carried on a hectic courtship between campaigns with the Army of Northern Virginia in which he served as a lieutenant in the Baltimore Light Artillery. Dr. Bean's boyhood and youth were spent under the most trying circumstances of economic distress in the backwash of the Reconstruction period following the war between the states. His formal schooling was intrusted to an aunt, Miss Regina Bean, whose teaching skill and discipline made a lasting impression. Despite the rigors of the times his home life was unusually happy and members of the large family were loyal and devoted. The Bible was a strong factor in his education and he retained deep religious convictions throughout his life.

From the age of thirteen until he entered the Virginia Polytechnic Institute at the age of twenty-two there was no opportunity for formal schooling. Experiences in farm work, clerking in a county store, managing the dining room at the old summer resort at Daggar's Springs, teaching school, mining, guarding convict labor, selling farm machinery and writing for a county newspaper gave a wide background of use-