THE SIGNIFICANCE OF THE BACTERIA AND THE PROTOZOA OF THE RUMEN OF THE BOVINE[1,2]

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CONTENTS

One of the most interesting chapters of biology, and probably one of the most neglected in instruction in biology, is that which considers the symbiotic relationship existing between living forms. The term "symbiosis", by derivation, means living together and usually carries the implication that each unit concerned in the relationship derives benefit therefrom and is necessary to the continued existence, in nature, of the associated units. For an informative discussion of the use of the word "symbiosis", the reader is referred to Calkins and Summers (1941, p. 890). The spatial arrangements are never included in the specifications but, by usage, the term is commonly restricted to those forms living in such intimacy that, so far as space is concerned, the associated forms comprise a unit, a family.

The symbiotic relationships existing between members of the world of microbiology and those of the world of macrobiology are among the most interesting and possibly the most important in this complex field of biology. This subject has been the field of interest of many students whose observations have been summarized by Paul Buchner (1930). Five of its 900 pages are devoted to the phase of which this paper is a part; namely, the relationships existing between the mammals and their associated microflora and microfauna. These relationships appear in the most evident form between the higher animals and the bacteria and protozoa of the alimentary tract, and especially between the polygastric animals and the flora and fauna of the upper part of the alimentary tract. If it is at all possible to arrange the symbiotic relations between mammals and their microassociates in the order of their relative importance to man, it is certain that those between the ruminants and the microörganisms would rank first. Yet less than one page of Buchner's review and fewer than 12 of the approximately 1250 references relate to this subdivision of the subject.

The subject has also been reviewed by Scheunert (1925), and by Scheunert and Schieblich (1927), and still more recently by Schwartz (1935). The latter review does not include a discussion of the mammals and their symbionts.

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Kirby has reviewed the relationships between certain protozoa and other animals in "Protozoa in Biological Research" by Calkins and Summers (1941). Fourteen pages are devoted to the ciliates of ruminants.

It is evident that the symbiosis of mammals and microörganisms has been studied much less than has that between the lower animals and the microorganisms. The explanation is evident: it is much easier to work with insects than with mammals.

I. THE RUMINANT

The most important to man's economy of the symbiotic relationships between mammals and microörganisms are found in the ruminants, the cud-chewing animals, in which the lower part of the esophagus forms a large sack, so large indeed that the food can be held therein for some 24 hours in the case of the bovine and from which it can be returned to the mouth for mastication. This arrangement permits the animal to ingest its food rapidly and to chew it thoroughly at a later time, a condition of great advantage to the animal in its feral state since during grazing it is more exposed to attack by its carnivorous enemies than at other periods. Hence the shorter the time that is required for the taking of food, the more likely it is to escape destruction. This arrangement also enables the ruminant to utilize certain kinds of energy-containing compounds to a much greater extent than can the non-ruminating herbivore. In most parts of the world the continuous growth of plants is not possible, due to sequence of seasons or to the alternation of wet and dry periods. Thus at intervals the herbivores must live on vegetation that has completed its cycle of growth, that has become dry, woody and resistant to the agents that degrade it into the simple products that can be absorbed from the alimentary tract. The species that can obtain the greatest amount of energy and nutriment from each unit of such material will not suffer the same handicap as will those that can obtain less benefit from it.

These two conditions seem to account for the fact that the ruminants predominate among the larger mammals in all parts of the world. They include all the cloven-hoofed animals other than those of the swine group. They are the most important that man has brought to his use as sources of power, clothing, and food. The world without the cow, the sheep, the goat, the camel, the water buffalo, the llama, the yak and the reindeer would be far different than it has been or is. Without domestic and wild ruminants nothing like the present population of the world could be fed, for a large part of the yearly crop now used to produce food for man would be returned to the soil as it is formed by the plant, or it would be used by organisms that cannot be eaten by man or, at least, are not now so consumed. The greater part of the organic matter built by the green plant consists of carbohydrates. The simplest and most soluble of these, the sugars, can be used by man and all the higher animals, The somewhat more complex and insoluble starches can be used by man and by most higher animals. These classes of compounds make up but a small part of the mass of matter constructed by the green plant. The more complex celluloses and lignins, wood substances, are much more abundant and are used by only a part of the higher animals.

Before any of the carbohydrates occurring in nature in any significant amounts can be used by the animal they must be so changed that they can pass into the blood stream and be utilized by the tissues. These changes are occasioned primarily by the various enzymes which are formed in the tissues and then excreted into the alimentary tract there to carry on their work of preparing the ingesta for absorption. No one of the mammals is known to produce an enzyme that will change cellulose and lignin into soluble compounds, but among the microbial associates of many animals are found, in some part of the digestive tract, forms that can produce enzymes that attack these compounds and prepare them for absorption. It is difficult or impossible to demonstrate these enzymes, as those that act on starch can be demonstrated in the liquid of the tract. It may be that the action is due to the intimate contact of the living cell with a particle of the compound, and that in some manner the material at the point of contact is made soluble in the menstruum at that point and thereupon pass into the cell. This has been demonstrated by Vogler and Umbreit (1941) for sulphur which is used by certain of the bacteria of the soil and by this action is made available for other forms of life.

It is commonly supposed that the enzyme is formed in excess of the needs of the microörganism and that the excess of the decomposition products, such as glucose from cellulose, is then available for the animal. It seems more likely that the action is so largely a contact phenomenon, that the host has opportunity to use these compounds coming from the degradation of cellulose by the microorganism only in the form in which they are built into the microbial cell. The result is the same so far as the host is concerned, for in either case whether the enzymes are of intrinsic origin or are formed by bacteria the material ingested is prepared for absorption.

It is commonly supposed that a larger part of the energy of the food is available to the animal when the enzymes are formed by it rather than by some other form that is living on the ingesta. It may be, however, that it makes little difference in the energy relations where the enzyme is elaborated. The animal and its associates may comprise a true digestive or physiological unit.

1. *The rumen and its rôle.* The term "rumen" as used in this paper includes the rumen proper or paunch, the reticulum or honeycomb, and the omasum or manyplies, the latter names giving some idea of the structure of these organs. The rumen is by far the largest of the three. The essential rôles of the other sacculations of the esophagus are not well understood and hence it seems proper to class all together so far as our discussion goes. The rumen of the bovine is slightly developed at birth. It slowly increases in size relative to that of the true stomach under the stimulus of solid food, especially of the type available to the animal in its native state. Such material in modern terminology is called roughage. The rumen will develop in the absence of such material, as for example, when only ground grain is fed which would contain only a small amount of cellulose and lignin. The writer has seen no statement as to whether the

rumen will develop if only milk is consumed. It has been stated that it will be reformed if removed by operation. In the adult bovine, the capacity of the rumen is such that when filled its contents will make up about one-fifth of the total weight of the animal. The rumen of an animal weighing 1000 pounds has a capacity of about 25 gallons.

The size of the rumen and the fact that the liquid therein contains no enzymes elaborated by the animal have caused students of the nutrition of the ruminant to look on the rumen as a storage chamber in which the food is kept until there is opportunity for its proper mastication and salivation which prepare it for digestion in the glandular stomach and in the lower levels of the tract. This view of the rôle of the rumen is still prevalent as is reflected in the following quotations from the volume entitled "Nutritional Physiology of the Adult Ruminant" by Ritzman and Benedict, (1938): "The paunch is a large warm storage vat that secretes no digestive juices", p. 7; "The function of this organ is of a purely mechanical character", p. 20; "That the greater efficiency of the ruminant in digesting coarse fodder is due to provisions of a mechanical rather than of a chemical nature appears to be well demonstrated experimentally", p. 8.

The incorrectness of this view is at once evident to one who views a drop of the liquid from the rumen under high magnification and thus is made aware of the abundance of microscopic forms living in the rumen. The protozoa and bacteria represent a density of population that is never met *in vitro* and probably not elsewhere in nature. This population has arisen from the material ingested by the animal and to think that this microbial population has no rôle in the economy of the animal is possible only for one who has had no training in microbiology. It would seem that much of the research and some of the feeding practices would never have arisen had the viewpoint of the microbiologist been considered. For example, until recently it had been thought impossible for the ruminant to care for any part of its need for nitrogen from urea, a waste product of the metabolism of the animal. It is now known that such is possible. The bacteriologist could have answered the question from his knowledge of the ability of many bacteria to utilize this source of nitrogen in the presence of appropriate sources of energy, and from his knowledge of the abundance of bacterial growth in the rumen. But he could not have answered the question as to the part of the total needs that could be derived from such a source.

2. *Conditions in the rumen.* W. B. Cannon has discussed in his book "The Wisdom of the Body"(1932) the ways and means by which a condition of constancy is maintained by the body in its various parts. If one considers the rumen a true organ, he would expect to find therein the same constancy characteristic of other organs. It is evident that the temperature therein will be that of the species in question, in the bovine about 39 C (102 F). This will aid in maintaining a constant type of microbial population. The usual food of the ruminant is such that the carbohydrates from which acids are formed by bacteria are in greater abundance than are the proteins from which alkaline substances are formed. Hence the reaction of a fermenting mass of plant material is likely to become acid in reaction, so acid indeed that further growth of

the bacteria is impossible, as in such self-pickled products, silage and sauerkraut. The same inhibiting action would take place in the rumen were there not provisions to prevent it.

The saliva of the bovine carries no enzymes. It does contain about 0.9 per cent of solids of which over one-half is inorganic matter, largely sodium bicarbonate. Man secretes about 10 ml of saliva per pound of body weight per day; and the fowl about 4 ml. The bovine secretes about 45 ml of saliva for each pound of live weight per day. Thus an animal weighing 1000 pounds secretes about 45 liters each day or about 90 pounds. The saliva has a pH of about 8.2. The neutralizing power of this great volume of saliva is evident By this means the reaction of the rumen is kept at a point that is favorable for the growth of bacteria and protozoa, just below pH 7. It seems probable that the ingestion of acid food such as silage causes an increased flow of saliva so that the constancy of environment so essential to the proper functioning of any organ is maintained. The saliva has additional rôles such as the lubrication and moistening of the food both at the time of its ingestion and later when it is cudded.

The oxidation-reduction conditions are such that only anaerobic organisms or the more anaerobic of the facultative ones can grow. Under the anaerobic conditions at which growth must take place in the rumen, there is very little release of energy as heat in spite of the enormous transformation of matter that is taking place. This loss is inconceivably small to one accustomed to thinking in terms of aerobic processes.

The wall of the rumen is constantly passing through a series of rhythmic contractions and expansions of such a nature that the food follows a rather definite path, at least any particular kind of food such as dry fodder tends always to follow the same path. The contraction of the walls of the rumen produce a constant mixing of the contents thereof and cause the lighter parts that are found in the upper part of the rumen above the level of the liquid to be constantly sprayed with the liquid, thus tending to remove to the lower layers all small particles and any part of the solid made soluble. A very large volume of gas is formed, which as it passes to the upper part of the rumen aids in the mixing of its contents. This gas is passed to the exterior through the esophagus and if, for any reason, the opening thereof becomes clogged a condition known as bloat or hoven results, which if not relieved causes the death of the animal. Many of the microörganisms of the rumen are motile and aid in the mixing of its contents. The result is that no part of the contents becomes stagnant and that the environment is controlled throughout the rumen.

The resultant of all of these factors of control is an environment constantly favorable to the reproduction and persistence of certain protozoa and bacteria. Fresh supplies of food are arriving at more or less regular intervals. Irrigation by saliva and water maintains a constant reaction and removes the products formed, as well as a part of the microörganisms, to the lower levels of the rumen from which they pass into the lethal environment of the glandular stomach, there to be digested and made ready for absorption. The transformation of food into microbes is so great that some have been led to say that the ruminant lives not on the material ingested but upon the mass of microörganisms that has been formed from that material. This statement is certainly more nearly true than one would infer from the modern literature on the digestive processes of the ruminant.

3. *The esophageal groove.* The lower part of the esophagus is a slit tube. The edges of the slit can be brought together if the food is to be passed to the omasum and thus to bypass the rumen proper; or the slit may be opened, in which case the food will enter the rumen there to be exposed to changes that will decrease the energy content from that which the food would have had if it had escaped such exposure. The very young animal lives exclusively on milk which can best be utilized if it passes directly to the true stomach. All seem to admit that the groove is under the control of the young animal and that the degree of control will depend on the position of the head as the milk is taken, being greatest when the head is in the suckling position and less perfect when in the drinking position. Thus some advocate that calves be fed from an artificial udder rather than from a pail. It also seems that the rapidity with which the milk is taken is of significance. If it is taken slowly, it will bypass the rumen, while if it is taken rapidly, much of it will enter the rumen.

Schalk and Amidon (1928) from a long series of observations on both young and adult bovines with artificial fistulae in the rumen conclude that the adult has little control over the esophageal groove and that saliva, water and all food enter the rumen. Ritzman and Benedict (1938, p. 288) state that in the case of the adult bovine only mucus is passed by the rumen and reticulum to the abomasum. They also state that concentrates such as meal do not enter the rumen. It is to be remembered that the domestic bovine ingests much material of a nature quite unlike that which it ingests in a wild state. It now receives considerable quantities of grain, either ground or unground. It seems clear that, while such finely divided material may enter the rumen, it remains there but a relatively short time, for the time any material remains in the rumen seems to depend on its density, heavy material being passed on much more rapidly than the lighter and more fibrous material that forms the main bulk of the food for the wild ruminant. The entrance of the food into the rumen or its bypassing thereof is important; so also is the time it remains therein. The shorter the time, the less will it be robbed of energy by the microbial process to which it is exposed.

It is evident that saliva must be passed into the rumen since otherwise it could not exert its buffering action, its most important rôle. The bolus swallowed after rumination is returned to the rumen but apparently quickly passes to the omasum.

Water taken in large quantities must either bypass the rumen or remain therein but a short time. The liquid passing out of the rumen must tend to carry with it the soluble matter and also that most finely divided, the extreme of which will be the bacteria. This is indicated by the observations of Mills *et al.* (1944) regarding the conditions under which urea is used by the bovine.

The results tend to show that starch is more effective than sugar in the form of molasses as a source of needed energy if urea is to be changed to protein. This difference may be due to the fact that the starch is retained in the rumen and becomes a source of energy for the bacteria that are building protein from the urea while the sugar passes beyond their reach with such rapidity that they cannot make full use of it.

The average loss of digestible energy, or that part of the total energy of the food which is used by the animal, in the form of methane produced in the rumen is, according to Ritzman and Benedict (1938), 11 per cent when a diet of hay, grain and silage is used. It is probable that hydrogen is formed by bacteria from carbohydrates in the rumen, and that in the environment of the rumen CO_2 is reduced by the hydrogen to form CH_4. Apparently not all of the hydrogen is thus combined at all times, so it is found occasionally in the gases of the rumen.

Other energy-containing gases are also produced in small amounts, such as hydrogen sulphide and carbon monoxide. These may be absorbed and subsequently excreted in the exhaled air. Some believe they are produced in larger than normal amounts in case of bloat and produce symptoms of toxicity. The addition of elemental sulphur to the feed will cause poisoning through the production of H_2S. It is evident that if the material ingested is of a nature that can be digested in the true stomach, much is gained if it does not pass into the rumen since it will be protected then from this 11 per cent loss. In the case of material such as cellulose, which cannot be handled by the true stomach, the energy made available to the animal will be greater if the material enters the rumen than if it does not. From the standpoint of economy of utilization of energy, the animal should have some control of the path of the food. This is apparently more important under domestic than under feral conditions in which the main mass of the food will be best utilized if it enters the rumen.

4. *Rumination.* The content of the rumen is kept in constant motion through the rhythmic contractions of the wall. These must be of such a nature that the various parts of the food will yield the maximum of value to the animal, and all parts of the same class of food will be retained in the rumen for much the same length of time.

At intervals a mass of the fibrous material in the rumen is forced up the esophagus to the mouth where it is masticated for about one minute. The stimulus for the raising of the bolus is apparently the irritation of the wall of a certain part of the rumen by solid, rough feed. It is often stated that the bolus is thus saturated with saliva, becomes heavy and is prepared to pass to the omasum. It is difficult to see how the squeezing to which the bolus is subjected in the chewing can do other than remove water from it with the result that it will become lighter rather than heavier, in spite of which it seems reasonable to suppose that the bolus just ruminated soon finds its way to the true stomach. Otherwise there would be disorder and inequality of treatment. The cud is not from the food most recently ingested, but from that which has been in the rumen for 12 to 24 hours. It has been exposed to the action of bacteria and is probably so changed that the mastication is much more effective than at an earlier stage. Microscopic examination of the rumen contents shows much more disintegration of the plant tissue than could result from purely mechanical action.

The average weight of each cud or bolus is about 100 grams, the average time of mastication about one minute. Since about one-third of the time is spent in rumination, it follows that about 48 kilos of moist rumen contents are ruminated in each 24 hours, and that the weight of the dry matter involved will be at least half of that ingested in each 24 hours. Dukes (1942) states that a bovine makes about 43,000 motions of the jaw per day in the chewing of the cud. Considerable pressure must be exerted in the process of mastication and the energy demanded must be great. Ritzman and Benedict (1938) estimate it at 11.3 per cent of the total energy in the case of hay.

There are grinding processes at work other than chewing. The constant motion of the contents of the rumen tends to tear the plant tissue apart. The writer has noticed a protozoon swimming in a circle and in each passage rubbing against the particles of food with such force as to cause a distinct depression of its cell wall at the point of contact. Calkins and Summers (1941, p. 979) describe and picture the comminuting action of certain of the protozoa of the rumen. Some seem to tear fibers away. Others bite pieces off. Some ingest large, relative to the cell, flat pieces of tissue and still others take in and roll up large cellulose fibers. Starch is digested and glycogen accumulates in the cell. Cellulose-splitting ability by protozoa of the rumen is questionable, Calkins & Summers (1941, p. 982). The food is also ground between the leaves of the manyplies. One may get some idea of the comminuting effect of these processes by imagining what would happen to cotton and linen cloth kept in a washing machine for 24 hours in the presence of organisms able to decompose the cellulose.

The value of rumination is probably shown in the observations of Mead and Goss (1935), who raised calves from birth to 18 months of age on a roughage-free diet consisting of ground grain. In one comparison a finely ground mixture was compared with the same mixture coarsely ground. The animals ruminated on the coarsely ground feed, but not on the finely ground. The results were opposite to those most would expect in that the average weight of the dry feces represented a higher percentage of the feed consumed in the case of the finely ground than in the case of the more coarsely ground as is shown by the following data:

Dry Feces as Percentage of Feed Consumed

COW NO.	COARSE FEED	FINE FEED	DIFFERENCE
34	21.0	19.7	−1.3
35	18.4	21.8	+3.4
37	19.3	21.1	+1.8
39	20.0	23.2	+3.2
Average	19.7	21.5	

The crude fiber on the average in the feces from the coarsely ground mixture was 21.2 per cent, from the finely ground, 22.9 per cent. Apparently the mastication was more effective than the grinding in making the crude fiber available. It is probable that the mastication was aided by a longer period in the rumen in the case of the more coarsely ground than in the case of the finely ground feed.

It has been shown that the animal masticates the feed more completely if it is silage alone than if it is silage plus corn meal, and that the extent of mastication decreases as the ratio of grain to silage is increased. Here as elsewhere in considering the question of utilization of feed, three conditions are involved: Does some part of a particular ration bypass the rumen? If a part of the feed enters the rumen, is it returned to the mouth for chewing? If a part of the feed enters the rumen but is not returned to the mouth, will the effect of its presence in the rumen for a considerable period be a negative or a positive one so far as utilization is concerned?

It is known that one-fourth to one-half of the whole dry corn ingested is voided in the feces, no use whatever having been made of the grain which is resistant to all the digestive juices of the tract. This can mean only that this part of the grain bypasses the rumen or that it is never returned to the mouth. The significance of size of particle is apparently not well known. For example, to what extent will a corn grain be digested if it is broken into two equally sized pieces which are not masticated? Apparently the digestive mechanism of the ruminant is not as well adapted to the handling of relatively resistant materials, when these escape mastication and treatment in the rumen, as is that of the nonruminating herbivore. Unground grain and very finely ground grain may be less well utilized by the bovine than that which is just cracked or very coarsely ground. It is evident that the student of feeding faces a most complex problem when he attempts to study the value of any ration.

II. FLORA AND FAUNA OF THE RUMEN

The microörganisms of the rumen of each species are apparently characteristic and are derived from the adults with which the young animal is associated at the time of the development of the rumen. The complex of life in the rumen must vary from one species to another, since the environment is not identical in the different kinds of animals; for example, the reaction of the rumen in the sheep is said to be slightly alkaline rather than slightly acid as in the bovine.

The native flora and fauna are supplemented by those of the food which may on occasion carry a great number of microörganisms. Thus, the writer has found over 50,000 actinomycetes in each milliliter of the rumen liquid of a cow being fed dry fodder. These soil organisms, being aerobic, could not have grown in the rumen and must have been ingested. The writer has also found similar numbers of a film-forming yeast that was ingested with silage. Under certain conditions such an aerobic organism will be growing in the upper layers of silage and will then be ingested in great numbers, and if its real origin is not recognized it will be classed as one of the intrinsic elements of the native flora of the rumen. The writer has also isolated from the rumen a rare form of acid-destroying bacteria which for a period was thought to be an intrinsic part of the flora, since it was facultative in nature. It was finally found to be carried in the silage in the upper layers of which it was growing. Thus, both water and food may be so heavily laden with microörganisms as to cause confusion in the mind of the analyst of the rumen contents. These adventitious forms are apparently of no significance. It seems probable that many of the forms of life reported as having been found in the alimentary tract are those from the outside rather than intrinsic forms. The appearance of plate cultures made on the usual media of the laboratory gives the impression that most of the forms came from the environment of the animal; much the same picture could be obtained from water, soil or air. The cultural methods reveal but a minute fraction of the total revealed by the microscopic examination of the untreated liquid of the rumen, again an indication that the majority of the forms present are peculiar to that environment. Our inability to cultivate them is due to the lack of knowledge of the chemical environment which permits their growth.

The continued existence of the native population of the rumen is made possible by the fact that the rumen never becomes completely empty and by the further fact that the environment is so mild that even though no growth may be possible for a period, the organisms remain not only viable but of high vitality and respond at once to the arrival of food. The rapidity of this response is shown by the rate at which gas is formed following the ingestion of food. This has been measured by a number of investigators and found to reach the maximum rate within an hour after the food is taken, even when such a resistant material as dry alfalfa hay is taken. The lag so noticeable when the bacteria of the laboratory are placed in contact with a fresh supply of food is striking by its absence.

1. *Protozoa of the rumen.* The most evident organisms revealed by the microscope in the liquid of the rumen are the protozoa, because of their relatively large size and great motility. Becker of the Iowa State College has been the principal American student of the flora of the cow, sheep, goat and horse. He and his associates have studied both the qualitative and quantitative aspects of the protozoa and also their physiological rôle in the economy of the animal.

The rumen fauna appears as soon as roughage is ingested, apparently due to the close association of the young with the adult of its species. After the ruminating stage is reached, each cud brings to the mouth a mass of the protozoa growing in the rumen thus to contaminate all the surroundings of the animal and to assure the quick and easy passage to the rumen of the young animal.

Each species of ruminant is supposed to have its characteristic fauna, some elements of which are found in the rumen of other species but not the entire complex. The report by Becker and Talbot (1927) on their examination of the rumens of 26 cows states that the greatest number of times a single kind of protozoan was found in the series was 23. This does not seem to reflect the probable condition in nature. The history of the animals previous to slaughter was not known and the varied treatment may have influenced the results. Recognizing that any invading mixture of protozoa coming from another animal finds in general the same environment as that from which it came, the conclusion of a characterisitc fauna seems inevitable. Calkins and Summers (1941, p. 924) state "that any termite of a species, wherever obtained, will be found to have the same group of flagellate species. Sometimes one or more flagellates are absent, but uniformity in composition of the fanules is the rule". The quantitative relations may vary so widely as to make the demonstration of each of the intrinsic types at each examination difficult without the expenditure of a great amount of time.

The inherent fauna will be supplemented by that of the feed and the water. These forms cannot grow in the rumen but may persist in the mild environment they there meet. The quantitative relations of the different inherent forms will undoubtedly change with marked changes in the feed as from dry fodder to fresh grass. Apparently no detailed studies have been made of these changes and it may well be doubted whether they are of much significance.

The larger forms of protozoa of the rumen are those that ingest particulate matter such as bacteria and small particles of plant tissue. In every sample feeding protozoa are seen, their waving oral appendages causing currents to flow by the oral groove and thus to bring within their reach the particles of food. The total effect of these currents in the mixing of the rumen liquid must be considerable. Soon after the ingestion of food by the animal, the protozoa can be seen filled with the granular matter they have ingested. Thus, when fresh grass is consumed the cells may appear quite green because of the chlorophyll grains ingested. Some of the larger forms have internal skeletons of silica which, together with other elements of a complex structure, seem strange in an organism classed as a unicellular one. The protozoa of the rumen are anaerobic as is shown by observations made by the writer. In a hanging drop preparation motility ceases in about 15 minutes, while in a wet mount, a thin film of the liquid between slide and cover glass, the cells remain motile for hours. In the hanging drop the conditions are aerobic, and in the thin film they are anaerobic except at the very edge of the film.

It is probable that a part of the protozoa of the rumen live on soluble matter that must pass through the cell wall.

The chemical task accomplished by the protozoa of the rumen is wholly unknown. It must be kept in mind that they are anaerobic and, in order to secure the needed energy, must degrade a great deal of material just as is the case with the anaerobic bacteria. It is certain that the byproducts will be complex and will serve as food for other protozoa or bacteria in the rumen, with the result that little energy will be released as heat, and with the further result that a new mass of matter will be formed in the new cells. The quality of the new proteins, carbohydrates and fats is unknown but one may well suppose that it is as valuable as the ingested material, if not more so.

The number of protozoa may vary with the type of feed and with the interval of the feeding cycle at which the examination is made. These organisms are constantly reproducing at the rate of possibly four generations per day and are constantly being removed to the true stomach there to serve as food for the animal. It is difficult to give any numbers per millitliter that will be significant. The numbers reported by a number of observers range from 500,000,000 to one billion per ml. When one considers their size relative to the bacteria, it is evident that even the lower value given represents the synthesis of a large amount of matter in each feeding cycle. Attempts have been made to determine the total mass of protozoal matter in the rumen at a particular moment. Thus Mowry and Becker (1930) estimate that they make up 10 to 15 per cent of the rumen content, Mangold (1929) 4.4 to 8.7 per cent. Ferber and Winogradowa-Fedorowa (1929) estimate that they make up 5 per cent of the total volume and that they represent 20 per cent of the dry matter in the rumen. If any such values are valid, it is clear that the protozoa must be of great significance in the economy of the animal, especially when it is remembered that 20 pounds of dry feed per day is a fair ration for an animal of average size.

Becker and Everett (1930) carried out digestion experiments with normal sheep and with those that had been made free from protozoa by treatment with copper sulphate. They noted a better utilization of the feed by the latter, a conclusion that has not been confirmed by other observers to the knowledge of the writer. In the absence of protozoa, bacteria would represent the end of the biological cycle in the rumen. The energy of transformation from bacteria to protozoa under the anaerobic conditions of the rumen is probably not great. No one is in a position to say which is the better feed for the bovine, bacteria or protozoa. Nature seems to have determined that the ruminant will live to a large extent on each since both are constantly being carried into the true stomach, there to be killed by the acid and then digested.

No one can say what part of the bacteria is consumed by the protozoa of the rumen. According to Calkins (1938), a single cell of *Paramecium caudatum*, one of the protozoa of the bovine rumen, may ingest two to five million *Escherichia coli* in 24 hours.

Becker (1932) has suggested the following possible rôles for the protozoa in the rumen:

1. Make easily digestible material
2. Harmful
3. Harmless
4. Check growth of bacteria and prevent putrefaction
5. Aid in the utilization of cellulose
6. Aid in mixing the contents of the rumen

They probably do little to check the growth of bacteria since these are undoubtedly always sufficiently numerous to utilize all food available to them or at least when aided by those protozoa that secure their food as do the bacteria by passage through their cell walls. The byproducts of any one form can never accumulate in the presence of such a balanced biological system as that in the rumen. Hence a state of stagnation as is implied by the term putrefaction cannot occur.

The possibility of the rôle given under 5, above, is indicated by the work of Cleveland (1923) who demonstrated the similar rôle of protozoa in the termites.

Most of the detailed work with biological systems has been done under aerobic conditions, as for example, in natural surface waters where the same sequence of organic matter, bacteria and protozoa is seen. This cycle continues through the crustacea and the fish. Juday (1942) has recently emphasized the wastefulness of this cycle. Thus 97.5 to 99 per cent of the energy of the original material is dissipated by the time the fish stage is reached. It must be remembered that such a system is operating under aerobic conditions where the transformation of chemical energy to heat is much greater than is true under anaerobic conditions, in which it is difficult to demonstrate the production of heat except when immense amounts of material are being fermented, as occurs in some of the modern industrial plants using anaerobic processes for the production of such products as butyl alcohol and acetone.

2. *Bacteria of the rumen.* The microscope reveals the richness of the bacterial life in the liquid of the rumen. One notes a density of bacteria rarely if ever noted in the cultures of the laboratory. This is due, of course, to the controlled environment as to temperature, to reaction caused by the buffering effect of the saliva, to the arrival at frequent intervals of fresh food, and to the constant removal of the byproducts of any one kind of organism by others of the complex sequence of life, and through the removal of the cells by the constant stream of saliva and of water passing through the rumen to the lower levels of the digestive tract. The number of bacterial cells in each milliliter of the liquid of the rumen is expressed by billions. Some have estimated that 10 per cent of the insoluble matter in the rumen consists of bacteria.

Cultures from the rumen contents reveal no such numbers as can be seen with the microscope. One cell out of each hundred or thousand seen can be cultured. This condition has been noted in all studies of the flora of the digestive tract from mouth to anus. Thus MacNeal (1909) in his extensive work on the fecal flora of man could culture but one, on the average, out of each 3000 cells seen with the microscope. Similar results have been secured in the study of the numbers of bacteria in the feces of the bovine and of other animals. The conclusion usually drawn by the bacteriologist, with his implicit confidence in his culture methods, is that most of the cells in the feces are dead. They must have been formed largely in the lower bowel where no lethal agent for them is known to occur. It cannot be lack of food, for bacteria rarely die from such a cause, and the great increase in numbers that takes place after the feces are voided is proof that this necessity is not lacking. This explanation of the marked discrepancy between the numbers shown by the microscope and by any cultural method may seem reasonable to many, but will seem much less reasonable when applied to the contents of the rumen with its inert environment. The rapid response of the flora to the arrival of fresh supplies of food is proof of the great viability and the high vitality of the bacteria of the rumen.

A great variety of morphological types of bacteria is seen in the liquid of the rumen, some in great numbers and others in exceedingly small numbers. One undulating spiral has been seen in every sample examined by the writer; and the examination of scores of fields was, at times, necessary for its discovery. The great variety of physiological types in each morphological form makes it impossible to decide as to the frequency of occurrence of any one kind by a microscopic examination. The ability to cultivate only a very small part of those revealed by the microscope also limits the completeness of the picture that the bacteriologist can obtain of the bacteriology of the rumen. The environment of the rumen can be duplicated *in vitro* so far as temperature and reaction at the beginning of the growth *in vitro* is concerned. It seems most probable that the inability of the bacteriologist to cultivate and thus to obtain in pure culture a larger part of the bacteria of the rumen is due to the fact that he cannot create for them the chemical environment they demand. It may be that this environment is provided only by their associates as has been so brilliantly shown by Twort (1913) for one of the pathogenic organisms, that of Johne's disease in cattle, which has been isolated only by the use of a medium that contains an extract of a related organism. As was stated earlier, many of the forms obtained on culture media are identical with those that can easily be obtained from the environment of the animal. Their numbers are small in comparison to the total shown by the microscope. This is evidence that the major part of the flora in the rumen is of types confined thereto, as has been shown throughout the history of bacteriology for a large part of the kinds of bacteria found in the mouth of man. The presence of types that cannot have grown in the rumen, because of the anaerobic conditions therein, indicates again that the present available methods reveal but a most minor part of the types characteristic of the rumen.

The popular idea that the biology of the digestive tract of animals will vary widely with varying food is probably not well based, at least when the normal variations in food are concerned, such as is true with all domestic animals and possibly man. It may be that on such diets as are currently used by students of nutrition in experimental work, the flora may be changed from that which would be present if a normal diet were used. It does not seem probable that, in the ruminant at least, changes in diet will cause changes in the flora of the tract. It would seem that the carbohydrates, the proteins and the fats from different sources are so similar that the biology of the tract will remain relatively unchanged with the changes in diet that normally occur. The corollary of this conclusion is that any class of nutriment will be of much the same value if one admits that it will yield much the same kind of crop of microörganisms in the rumen. On this basis the comparison of different feeds, one of the favorite fields of research of the animal husbandman, seems quite futile.

The estimation of the total quantity of living matter in the rumen at any moment is impossible. The values given earlier may seem to many beyond the realm of possibility. That they are not so unreal as one may think follows from a comparison of the rumen liquid with blood. It is stated, Dukes (1942), that the corpuscles of the bovine blood make up about one-third of its total volume. The number of red cells per milliliter is about six billion, the average diameter is 5.6μ. Thus, in 1 ml. of blood there will be about 396 billion cubic microns of corpuscles. From values given by many there will be in each milliliter of the liquid of the rumen 100 billion cubic microns of protozoa and 15 billion cubic microns of bacteria. It is not at all impossible that 10 per cent of the volume of the rumen contents at the peak of a digestive cycle consists of bacteria and protozoa.

The point of interest is what this mass of living matter means in terms of ingested food. Hale, Duncan and Huffman (1940) state that with a ration of 20 pounds of alfalfa hay, 87.3 per cent thereof disappeared as such in the rumen, 84.5 per cent of the protein, 74.4 per cent of the cellulose, and 100 per cent of other carbohydrates.

Pearson and Smith (1943) conclude from their observations of the rumen contents (to which urea was added as soon as they were removed from the rumen and kept at 39°C for two to four hours), that at least 25 per cent of the total protein requirement of a cow yielding 25 pounds of milk per day can be synthesized from urea. From such observations it is concluded that the total transformation of nitrogen from all sources in the rumen to the protein of microörganisms may well approximate the total protein requirements of the animal.

3. *A balanced biology.* In such a controlled environment as the rumen provides the biota becomes balanced. No one unit of the system can get far out of line quantitatively, and hence there is no opportunity for the by-products of any form to accumulate and to reach a level that will be injurious to it or to other forms. If the continuity of the system is to be assured, this balance must be maintained.

It is very probable that some units of the system may be removed without disturbing its continuity for probably here as elsewhere in the mechanism of the body considerable compensation is provided. The observations of Becker and Everett (1930) indicate that the protozoa may be removed without serious disturbance of the physiological system of the host. This would seem to indicate that bacterial tissue is as available as food to the host as is that of protozoa. However, the time element in all such trials as those of Becker may have been so short that changes which may seem insignificant for the short period may become significant when a much longer period is involved.

It seems safe to conclude that the biota established by slow evolution in any species is attuned to the total system, that of the host and the associated microorganisms, and that man had best go slowly in judging the effects of any induced change in the biota of the digestive tract of any species.

One must think that the microörganisms of the rumen are building a mass of new organic matter from that ingested by the host, and that this is done by the transformation of the molecules without the loss of much energy. Thus the two molecules of alcohol resulting from the fermentation of glucose have almost as much energy as did the original sugar and the same is true if the glucose is changed to lactic acid. The quality of the new matter in relation to the nutrition of the host can only be surmised. It seems probable that it is equal in this respect to that ingested, if not superior thereto.

The vitamin requirements of any higher animal cannot be learned unless such animals can be kept, for a considerable part of the normal life span, free from microörganisms. Some students of nutrition believe that all the higher animals require the same vitamins and possibly in the same relative proportions. One can learn the kind and amount of vitamins that the food of any species of higher animal must contain for normal life. This is predicated on the supposition that the methods used in the assay of various foods yield the same values as do the metabolic processes of the species in question. If the animal flourishes on food not containing a full complement of vitamins, one must suppose, on the basis that all animals have the same qualitative if not the same quantitative requirements, that the deficiencies are made up through the elaboration of vitamins in the digestive tract by bacteria. It is not known that any protozoa are able to synthesize vitamins. The needed quantity of many of the accessory substances is so small that one can well imagine the formation of an adequate supply of one or more by the bacteria growing in the digestive tract.

It has been shown, Lardinois *et al.* (1944), that the following members of the B-complex are formed in the rumen of the bovine: thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid and biotin. It is, of course, also possible that they may be destroyed in the tract. Becker's observation, that sheep used the food more completely in the absence of the protozoa of the rumen than in their presence, may be correct when certain kinds of measuring devices are used, but it seems questionable that it is true for the total physiology of the host over long periods.

4. *A biological unit and its evolution.* One should think of the higher form and the associated biota of its digestive tract as a true physiological unit, and that the evolution of the symbiosis has taken place as has that of the physiology and the agents that carry on the chemical work of the body. If a particular element of the biota becomes of no value as the evolution progresses, it tends to disappear from the system, and if the need arises for a new element it will gradually appear.

In the field of enzymology one speaks of an adaptative enzyme, meaning thereby one that is formed under the stimulus of a substratum that has for the first time been presented to the organism. The organism adapts itself thereby to the new situation. May not the same be true in regard to the flora and fauna of the digestive tract? Indeed, it must have happened and be happening if evolution is to be accepted for all forms that have symbiotic relations; and apparently all animals do have such relations with some of the microörganisms in spite of the fact that a condition of sterility of the digestive tract has been reported for some of the animals of the polar regions. If the latter be true, some research foundation should, without delay, establish a laboratory there for the study of the nutritive requirements of those animals, for under such conditions some facts might be revealed that are out of man's reach working in the regions where such sterility of the tract is unknown.

5. *The cecum.* The discussion has been primarily concerned with the biology of the rumen of the bovine and its significance to this host. Other herbivorous animals have mechanisms that seem to serve much the same purpose as the rumen. Thus the large cecum of the horse provides a chamber in which the ingesta, after they have been acted on by the digestive juices of the upper part of the tract, are then exposed to the prolonged action of bacteria and of protozoa. The ability of the horse to digest cellulose is dependent on this mechanism. The mastication of woody material by the horse is less than that by the bovine because the horse has to chew the dry material, while the bovine chews the same

sort of material after it has been soaked, milled, percolated and fermented for a number of hours. The incompleteness of digestion of fibrous material by the horse and also by the rabbit and elephant is shown in the high cellulose content of their feces.

The large cecum of the rabbit plays a rôle similar to that of the horse. The biology of the ceca of such animals as the horse and the rabbit has not been studied to any extent.

6. *Coprophagy.* The subject of coprophagy is one to which little attention has been devoted in the study of the nutrition of animals. The eating of the feces is claimed to be a constant phenomenon in the rabbit. It is noted in rabbits kept on special diets and has been thought to be a consequence of the inadequacy of the diets which the animals attempt to overcome by consuming their feces. With modern techniques this consumption is supposedly prevented by keeping the animals on screens. Apparently this is less successful in the case of the rabbit than is commonly supposed, since the animal takes a part of the feces as they are voided, a process that will occur whether the animal is on screens or not. Apparently two types of feces occur, the hard type, the one that is evident, and a moist type that is consumed as voided and hence escapes notice. They are termed the day and night types respectively. Recent observations by Southern (1940) indicate that the swallowing of the moist feces is a natural phenomenon, not a reaction to an artificial environment as is so commonly supposed at present. It is interesting to consider how such a phenomenon has escaped notice until recently, especially in view of the domestic raising of rabbits, and of the quite constant presence of the moist type of feces in their stomachs. Such an animal as the rabbit utilizes a regenerative mechanism. Some by-products are returned to the system there to be used in the chemical processes of the body.

Coprophagy is also a natural phenomenon in the case of the normal rat, although here as in the case of the rabbit it is commonly supposed to reflect an abnormal and inadequate diet. It is not certain that this animal, as the rabbit, takes the feces as voided, since few observations have been made. Rats so restrained that feces cannot be reached should be observed. Without such observations the needed vitamin content of the food for this animal cannot be learned with any certainty. Coprophagy is practiced by many other animals, either throughout life or for certain periods thereof. Black (1942) has noted that rats on deficient diets prefer feces of normal rats to their own. He also says that coprophagy is always noted in experimental rats. It seems that the animal thus obtains certain substances not otherwise available to it and that these substances are contained in the feces, hence the attraction of the animal to them. When the final chapter is written on the ability of the animal to select its own food from a mixture, many students of nutrition will be surprised at the incompleteness of their knowledge. The instinct of the unhampered animal as a guide to its needs for food is often very surprising, and it may be that man would be as well off if he allowed his instinct to operate as to follow the advice so freely offered today for his guidance in selecting his diet.

7. *Digestion.* The term "digestion" is used in many ways in present-day

literature on nutrition. The physiologist bases his statements regarding the changes which food undergoes in the digestive tract on his knowledge of the inherent enzymes of the body and, of course, on those which he can separate and study *in vitro*. He is thus thinking of certain processes of degradation by which the food is so changed that it can pass through the wall of the intestine or be prepared for absorption. If some of the agents causing this change are derived from the microörganisms growing in the tract, it seems proper to include these agents as part of the digestive mechanism. Thus if an amylase of microbial or of plant origin functions in the digestive tract, there seems to be no reason why the changes produced thereby should not be included under the term. The synthesis of new material, such as takes place in the rumen for example, is quite a different matter, and it is questionable whether it should be included under the term "digestion" any more than if it went on outside the tract. Is the formation of alcohol or of lactic acid a phase of digestion? It would seem desirable to clearly differentiate between the synthetic processes and those of degradation in speaking of digestion in order that the mind of the reader be not confused. If one accepts the definition of digestion offered by Cannon (1936), namely, that it is the intimate process by which the food eaten is transformed from its natural, complex solid, semi-solid or unsuitable fluid state into a state of being soluble in the juices of the small intestine, then little if any digestion takes place in the rumen; while if one accepts the definition of Morrison (1936) namely, that all the changes which food undergoes within the digestive tract to prepare it for absorption and use in the body are known as digestion, one could consider the change of cellulose to bacterial tissue as it occurs in the rumen as digestion. As has been stated earlier, there is no reason to believe that appreciable absorption occurs through the wall of the rumen, since there are too many agents to consume immediately any food material made available and thus to use it before it can reach the absorbing surface which may be many inches away from the point of formation.

From statements frequently made by students of the nutrition of the bovine as to the use by the animal of the by-products of the microbial destruction of organic matter in the rumen, one would conclude that at certain periods in a digestive cycle, the percentage of soluble matter in the liquid of the rumen would be considerable. The writer (unpublished data) has determined the total soluble matter in the liquid of the rumen at various periods in a digestive cycle. The insoluble matter was removed by filtration through porcelain or by prolonged centrifugation. The values found approximated 1.5 per cent of soluble matter, a value not far different from those given for the soluble matter of the bovine saliva. In the case of the horse and rabbit the cecum seems to play, at least to some degree, the rôle that the rumen plays in the polygastric animals. The food that has not been digested in the stomach and small intestine enters the cecum, there to remain and be acted on by bacteria and protozoa with the building up of a mass of microbial tissue which must be digested and the soluble products absorbed in the large intestine, since the reasons against the absorption of soluble products of microbial action from the cecum are similar to those counter-indicating such absorption from the rumen.

In man, the bacterial content of the digesta reaches its lowest level in the stomach and the upper part of the small intestine, and its highest level in the large intestine. MacNeal (1909) states that about 46 per cent of the nitrogen excreted in the feces of man is contained in the cells of bacteria. Mangold (1929) says that 14.7 to 18.7 per cent of the dry matter of bovine feces consists of bacteria. It has also been shown that the bacterial content of bovine feces increases after the same are voided.

The bacterial content of the small and large intestines increases as lower and lower levels are reached. The usual explanation is that these bacteria are living on the undigested residues of the ingesta. This explanation seems inadequate since the food, especially in the ruminant, has been exposed to a series of active agents that would seem able to accomplish all that any of the bacteria of the lower levels of the tract could accomplish. It seems more probable that the food for such bacteria is the organic matter that is excreted into the lower part of the tract. The fact that there is no known food that will cause much decrease in the volume of the feces in man is indicative of this source of food for the bacteria of the lower bowel. Some years ago the writer observed young men who lived on milk alone for over four months. The only additional food taken was an orange or an apple each day. Theoretically the milk should be completely metabolized and the volume of the feces should be greatly decreased, when it represents almost the entire food consumed over a considerable period. This did not happen. The volume of feces was not markedly decreased from that on a mixed diet.

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