

Studies on the Alimentary Tract of Merino Sheep in South Africa. XI.—Digestion and Synthesis of Starch by Ruminal Bacteria.

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INTRODUCTION.

A low-power microscopic examination of rumen ingesta reveals that infusoria are the most conspicuous organisms. A more detailed examination, however, also shows the presence of large numbers of non-pathogenic bacteria, yeasts, fungi and spirochaetes, constituting the microflora of the rumen. The paunch and the large intestines of herbivores are large storage organs in which micro-organisms digest cellulosic structures and make available to the host animal previously encrusted food materials such as pectins, proteins and carbohydrates (Meyer, W., 1927). Meyer has shown that in this process the cell membranes first become swollen, then they appear lighter in colour and with progressive digestion the cellulose lamellae become torn, cavities are formed and disintegration sets in. The bacteria are seen surrounding the cells and later penetrating into the interior where the digestive process is continued. Baker and Martin (1937-1939) also investigated the disintegration of cell-wall substances in the gastro-intestinal tract of herbivora. According to them disintegration is evidenced by the formation of clearly defined zones of erosion with changes in microchemical and staining reactions associated with micellar disorganisation. Cellulose, hemicelluloses and pectic substances are dissolved while cutin and heavily lignified structures entirely resist attack. In the caecum of the guinea pig, the rabbit and the horse as well as in the rumen of the sheep, the primary agents of this disintegration were found to be iodophilic micro-organisms, giving a blue (amylodextrin) reaction with iodine. There is very little information to be found in the literature on the digestion in the rumen of the remaining foodstuffs, namely protein, fats and starch.

Mangold and Schmitt-Krahmer (1927) demonstrated bacterial digestion of fats in the rumen. Ferber (1928) showed that infusoria readily ingested fat droplets, and that these fats were digested within the organism by bacteria.

Baker and Martin (1933) observed that in the caecum of the horse there was a deposition of polysaccharides in the indigenous micro-organisms. The indication is, therefore, that these organisms had the ability to form starchlike polysaccharides within their own bodies, from the surrounding medium containing cellulose and other encrusted food materials, or their fermentation products.

Since no researches on bacterial digestion of starches in the rumen are recorded, this study was undertaken in an attempt to elucidate some aspects of this problem.

BACTERIAL DISINTEGRATION OF STARCH GRANULES WITHIN THE RUMEN.

Eight fistula sheep were placed on a wheat straw diet without any chemically detectable starch. On this diet the multiplication of starch digesting bacteria in the rumen could be controlled either by the addition or the reduction of starches or sugars.

The sheep were also freed from infusoria by copper sulphate administration some weeks before, so as to have all the starch administered into the rumen available for undisturbed bacterial digestion only.

Smears were made daily from rumen ingesta, withdrawn through the fistula, by adding a drop of Lugol's iodine to a drop of ruminal ingesta on a slide and covered by a coverslip. These preparations were then examined for starch granules. When no more granules could be seen, 5 grams of maize starch was administered into the rumen of each of four sheep at a time. After this, small samples (2 c.c.) of rumen ingesta were withdrawn hourly to follow up the process of disintegration of the starch granules. The preparation of smears was usually continued until the starch was completely disintegrated.

The mode of attack and disintegration of the starch granules is clearly illustrated in plate IV. Fig. 2 shows a few slightly mauve cocci adjacent to the starch granule, one hour after administration of the starch into the rumen. Fig. 3 shows a starch granule with double the number of mauve cocci around it, photographed one hour later. Fig. 4, taken three hours later, again shows an uncountable number of mauve and dark mauve organisms surrounding the starch granule. Fig. 5, taken seven hours later illustrates another granule with almost a pure culture of iodophilic streptococci surrounding it. The typical disintegration of a starch granule is well illustrated. It is excavated from the sides and there are bacteria filling these craters and enlarging them until the granule breaks up into fragments. In Fig. 6 the remnants of such a granule are seen after having been in the rumen for 24 hours. Only a mass of dark mauve and brown cocci is left. Some of these cocci are already losing their iodophilic reaction. Three or four hours later most of them will have become translucent again unless fresh starch granules or sugars are introduced.

Although in most instances there were numerous iodophilic organisms of various types present in the rumen, the disintegration of starch granules was mainly brought about by an iodophilic streptococcus usually present in large numbers. The disintegration followed a definite pattern.

By incubating sterilised starch granules with bacteria-free ruminal fluid obtained both by centrifugation and filtration (Zeitz filter and collodion membranes), disintegration of starch granules could not be produced. On the other hand, when incubated in a synthetic medium, similar to that used by Woodman & Stewart (1928) in their researches on cellulose digestion, with cultures of the iodophilic streptococcus isolated from the rumen, starch was attacked as in the rumen, without noticeable evolution of gas.

Furthermore, disintegration of starch granules was never observed in the rumen if the granules were not attacked by iodophilic bacteria, mainly streptococci.

It seems, therefore, that in the absence of diastatic enzymes in the saliva of the sheep, other diastatic enzymes were either not present in the ruminal fluid or otherwise too dilute to have any perceptible effect. Consequently the only diastases present in the rumen seem to be those secreted by ruminal bacteria, which would then be responsible for the entire starch disintegration seen here.

RATE OF DISINTEGRATION OF MAIZE STARCH GRANULES IN THE RUMEN.

This is influenced by the presence in the rumen of:—

- (a) A developed iodophilic flora.
- (b) An undeveloped flora.

(a) *Developed flora.*

To promote the development of a starch digesting flora 100 grams of maize starch was administered daily for 10 days through the fistula tube of a sheep on a hay diet. After this the rumen was prepared for starch digestion trials by omitting the administration of starch 48 hours before commencement of the trial. After this period the rumen had become free of starch and the organisms had all lost their iodophilic reaction. Under these circumstances the rumen thus contains a rich iodophilic flora ready to attack any starch that may be administered.

On the day of the trial 100 grams of maize starch was introduced through the fistula of each of three sheep, using different sheep in subsequent trials. A small sample (2-3 c.c.) of rumen content was withdrawn for microscopic examination at regular intervals of 30 minutes after administration of the starch. Microscopic preparations were made as in the previous experiment. The procedure was continued until neither starch nor iodophilic organisms could be seen in the preparations.

The results were briefly as follows:—

- (1) After 30 minutes a few iodophilic streptococci were present around most starch grains, some of the organisms staining light mauve whilst others were still translucent. Pseudo-yeasts [Fig. 5 (b), Plate 2] dispersed in the medium, stained a pale greenish-yellow with Lugol's iodine.
- (2) After one hour the number of organisms were notably increased and more of them staining mauve.
- (3) After 2 hours most starch grains were completely surrounded by iodophilic streptococci. A type of large iodophilic streptococcus forming rather long chains was usually visible from this stage onwards.
- (4) After 3 hours layers of iodophilic streptococci were packed around practically all the starch grains with streptococcal chains and groups of tetrads [Fig. 3 (b), Plate 2] dispersed throughout the medium.
- (5) After 5 hours disintegration of starch granules commenced.

- (6) After $6\frac{1}{2}$ hours disintegration of some starch granules was completed and iodophilic bacteria were dispersed throughout the ruminal fluid in large numbers [Plate 2, Figs. 3 (a), (b) and (c)]. Both pseudo-yeasts and the cigar-shaped bacilli (Fig. 5 (a), Plate 2] commenced to show iodophilic reactions at this stage.
- (7) After 18 hours only remnants of starch granules were present. These were surrounded by masses of streptococci, most of which had become translucent again. The iodophilic micro-organisms that were dispersed throughout the ruminal fluid were rare because they had completely metabolised the polysaccharides responsible for their previous iodophilic reactions.
- (8) After 20 hours there were neither starch residues nor iodophilic reacting bacteria. Starch disintegration was, therefore, completed in from 18 to 20 hours.

(b) *Undeveloped flora.*

For the purpose of these observations 8 fistula sheep on a hay diet without starch supplementation were used. Microscopic examination of their rumen ingesta revealed no iodophilic micro-organisms. The trials were carried out as in (a).

(1) After $1\frac{1}{2}$ hours, compared with 30 minutes in (a), translucent bacteria were seen in contiguity with the starch granules.

(2) After $2\frac{1}{2}$ hours the organisms reacted to iodine, i.e. $1\frac{1}{2}$ hours later than in (a).

(3) Disintegration of starch granules commenced after 7 hours, i.e. 2 hours later than in (a).

(4) Disintegration was completed after 26-28 hours. In one sheep complete disintegration took 36 hours.

Conclusions.

In the rumen of sheep receiving a regular supply of starch in the diet the bacterial disintegration of starch granules commenced after 5 hours and was completed within 18-20 hours, whilst in the case of sheep not receiving starch in the diet disintegration commenced after 7 hours; it was much slower and took 8-10 hours longer. In the sheep receiving starch regularly, the bacterial flora concerned in the disintegration of starch is well developed and consequently more organisms are available to attack the starch granules.

In the case of starch disintegrated by bacteria within infusoria it was shown above that disintegration commenced after $5\frac{1}{2}$ hours and was completed within 24 hours.

RATE OF DISINTEGRATION OF STARCH GRANULES OF DIFFERENT CEREALS.

Using the same technique as in the previous experiment, a series of experiments was conducted to determine the rate of disintegration of the various cereals that may be incorporated in the feeds of ruminants.

According to measurements taken by the author in the course of this investigation, starch granules of the various cereals were found to differ in size and shape, *vide* Table 10, below. Taylor and Iddles (1926) have found that there is also a difference in composition of the various starches. The sizes and shapes of the granules in a cereal also vary, e.g. potato starch granules may vary in length from 9.72 microns. Their shape is either spherical or oblong. The granules of maize, wheat, oats, rice and peanuts have a much more uniform spherical shape.

In the table given below it is shown that starches with large granules are digested more slowly than those with small granules. Potato starch which has the largest granules, requires 11 hours more than oats which have the smallest granules. The average maize starch has $\frac{1}{3}$ the size of the average potato starch granule and is digested 10 hours sooner than the latter.

TABLE 1.

Comparative diameters of starch granules in microns and rate of digestion.

Starch.	Largest granule.	Smallest granule.	Average granule.	Time required for complete digestion.
Potato.....	72	9	35	28-30 hours.
Wheat.....	39	6	24	24-26 ..
Maize.....	18	3	12	18-22 ..
Peanut.....	15	3	9	18-22 ..
Rice.....	15	3	6	17-20 ..
Oats.....	12	3	6	17-20 ..

From a statistical analysis of the diameters of the different types of starch granules and the rate of digestion, a linear relationship was found to exist between the log. of the time and the diameter of the granule. The diameter of starch granules, therefore, definitely influences their rate of disintegration in the rumen.

THE DIGESTION OF STARCH WITHIN THE RUMEN.

Degradation of starch usually results in the formation of soluble carbohydrates like dextrins and sugars (Blake, 1946; Starke, 1942). It has been shown above that some of the products of starch degradation are resynthesised into glycogen and starchlike polysaccharides in certain ruminal micro-organisms. The polysaccharides thus formed are utilized by the organisms for their own metabolism as evidenced by the active division seen in the pseudoyeasts during these stages.

As weak organic (fatty) acids, e.g. acetic and lactic acid have been found to be usually present in the rumen (Woodman, 1927) it is possible that these acids are fermentation products of some of the soluble carbohydrates formed during the process of starch hydrolysis. These acids are in turn changed into salts, e.g. acetates and lactates owing to the presence of large amounts of carbonates in the rumen.

The soluble carbohydrates are, therefore, partly resynthesised into glycogen and starchlike polysaccharides and probably also partly changed into weak organic acids and their salts. The percentage of starch formed

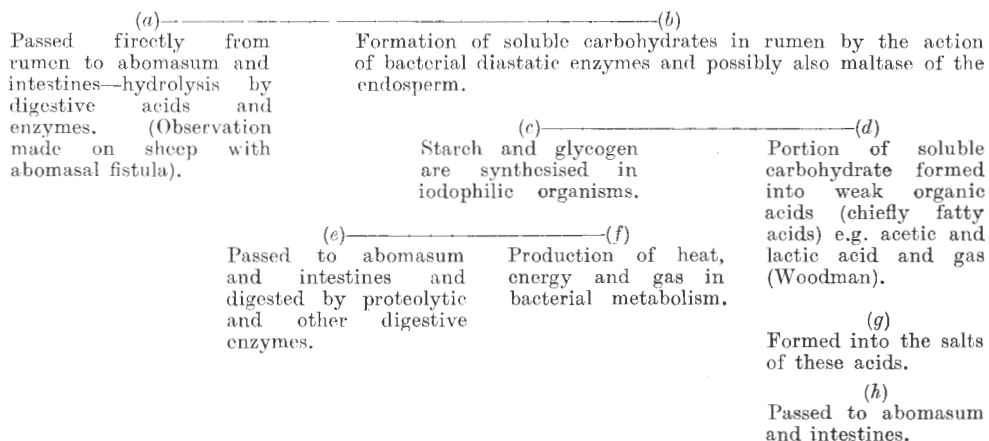
into organic acids is unknown. According to Woodman only about 8 per cent. of the total digestible carbohydrate of the food in the rumen is broken down to the gas and organic acid stage. He assumes consequently, that approximately 8 per cent. of the sugar present in the rumen will undergo further bacterial breakdown, and that the rest of the sugar will be absorbed in the alimentary tract of the animal. Woodman advanced these arguments to support his theory that cellulose is broken down by bacterial digestion to cellobiose and thence to dextrose. He considers this comparable to the digestion of starch which is first hydrolysed to maltose, and then to dextrose, by enzyme action. Woodman's theory is, therefore, that all carbohydrates in the rumen are ultimately broken down to dextrose and absorbed as such excepting for a possible 8 per cent. which are broken down by bacterial action to organic acids and gases.

Mangold (1929) opposes Woodman's theory and suggests that in the rumen, substances such as sugars, starches, and cellulose, ferment to form gases, organic fatty acids and alcohols. Neither Woodman nor Mangold, however, considered the possibility that the microflora may be able to reform starchlike polysaccharides and glycogen from the breakdown products of starches introduced into the rumen. They regarded the micro-organisms only as secretors of hydrolytic enzymes assisting in the breakdown of organic matter in the rumen.

The amount of digestible carbohydrates formed into acids and gases in the rumen is difficult, if not impossible, to assess, because material is constantly passing out of the rumen, to the omasum and abomasum, particularly during feeding (Quin and van der Wath, 1938). It is thus impossible to calculate at any one moment, how much of a substance placed in the rumen at a given time is still left in it at a later stage. Under these experimental difficulties it is, therefore, impossible to evaluate the amount of starch digested in the various ways in which it may be dealt with in the rumen.

A schematic presentation is given below of ruminal digestion of starch, based on the data obtained in this study as well as on some known facts about starch hydrolysis.

STARCH ADMINISTERED INTO THE RUMEN.



TYPES OF RUMINAL IODOPHILIC MICRO-ORGANISMS AND THEIR REACTION TO
ALCOHOLS AND CARBOHYDRATES.

In studying the digestion of starch in the rumen several types of iodophilic micro-organisms were observed when sheep were fed either starches or sugars. Baker and Martin (1937) described the iodophilic organisms encountered in a study of cellulose digestion in the caecum of the rabbit. They found similar organisms in the caecum of the horse and guinea pig and subsequently (1938) also in the rumen of sheep. They concluded that the primary agents in the disintegration of cell wall substances in the rumen were iodophilic micro-organisms very similar to those observed in the horse, guinea pig and rabbit. By using special methods these investigators claim to have observed iodophilic bacteria in the interior of lacunae formed in the cellulose particles. The iodophilic bacteria and pseudoyeasts concerned in the digestion of starch in the rumen can be demonstrated without difficulty by using Gram's or Lugol's iodine. To a drop of ruminal fluid on a clean slide a drop of Gram's iodine is added and covered with a thin coverslip. Any iodophilic organisms, whether brown or blue, are immediately discerned.

The bacteria and pseudoyeasts enumerated below (Table 2) were observed in the rumen of the sheep used in these experiments. Apart from their size and physiological action detailed descriptions of these organisms will not be given. They are clearly illustrated in photomicrographs Nos. 1 to 5, Plate V.

TABLE 2.

Micro-organisms.	Size. (microns).	SYNTHETIC PRODUCTS.	
		Glycogen.	Starch.
Streptococcus (Photo 1, Plate V).....	·65	—	×
Streptococcus, Large Type. (Photo 2, Plate V)...	1·35	—	×
Tetracoccus (Photo 3b, Plate V).....	5×5	—	×
Small bacilli—various types (Photos 3c and 5, Plate V).....	{ 5×3 5×2	×	×
Large iodoph. cigar-shaped bac. [Photo 5(a), Plate V.].....	{ 2·5×16 4×13	—	×
Pseudo-yeasts, dividing by binary fission (Photo 4 and 5b, Plate V.).....	4×8	×	—

In the course of this study it was observed that these iodophilic organisms reacted differently to different carbohydrates, e.g. starch and glucose. If glucose was administered into the rumen, the pseudoyeasts and some bacteria, especially the cocci, soon reacted iodophilically by staining dark brown and blue, respectively. When starch was administered these organisms reacted similarly, but the reaction was much delayed and not as strong. It was, therefore, decided to test the reaction of the iodophilic flora to the introduction of various alcohols, sugars and other carbohydrates, into small quantities (10 c.c.) of ruminal ingesta incubated at 39° C. immediately after withdrawal from the rumen. The test material was used on concentrations of two and five per cent. in the ruminal ingesta.

Microscopic preparations stained with Lugol's iodine were made and examined after 15 minutes, 45 minutes, 3 hours and in some instances 20 hours after the commencement of incubation. The observations on starch were carried out *in vivo* at the same time and on the same sheep.

The results are tabulated in Table 3.

It will be observed that:—

- (1) The three pentoses used, namely arabinose, xylose and rhamnose were not assimilated by the micro-organisms to form iodophilic polysaccharides. The other monosaccharides used were hexoses. Of these dextrose (glucose) and laevulose (fructose) were readily assimilated and condensed into iodophilic polysaccharides. Galactose, however, produced an iodophilic reaction in the cigarshaped bacillus only.
- (2) The disaccharides maltose and saccharose were assimilated by all types of iodophilic micro-organisms, but not as fast as the laevulose and dextrose. Lactose and cellobiose produced delayed iodophilic reactions in the bacteria only.
- (3) The bisaccharide, raffinose, produced an early iodophilic reaction in all types of iodophilic organisms.
- (4) Amongst the polysaccharides, starch and pectin showed delayed reactions in the bacteria and to a lesser extent in the pseudo-yeasts as well. The readily soluble polysaccharides inulin and soluble starch were assimilated within 15 and 45 minutes respectively.
- (5) Of the alcohols, isodulcitol produced no iodophilic reaction, whilst mannitol and sorbitol were assimilated soon, although the last-named was apparently poorly assimilated by bacteria.

Conclusions:

- (1) Of all the substrates used, laevulose (fructose) and dextrose (glucose) were most readily assimilated by the iodophilic flora, as judged by their intense iodophilic reaction.
- (2) Starch was metabolised, but very slowly, probably owing to its insolubility and complex molecular structure.
- (3) The pentoses did not produce iodophilic reactions in any of the ruminal micro-organisms. These substances have been shown not to be fermented by yeasts (Plimmer, 1940).
- (4) Cellobiose, derived from cellulose, produced a delayed iodophilic reaction.
- (5) Some alcohols are assimilable by the ruminal micro-flora.

It appears, therefore, that the micro-flora of the rumen can assimilate a wide range of carbohydrates. The rate and extent of assimilation is in some measure determined by the complexity of the molecule. This ability of the ruminal micro-flora is of great advantage to the host animal as the great variety of complex carbohydrates naturally occurring in its diet can be hydrolysed to less complicated structures, and made available to the host animal in a more assimilable form.

TABLE 3.

Reaction of Ruminal Iodophilic Micro-organisms to Alcohols, Sugars and Other Soluble Carbohydrates.

Substrate.	Nature of Substrate.	PERIOD OF INCUBATION AND REACTION OF ORGANISMS.			
		15 minutes.	45 minutes.	3 hours.	20 hours.
Galactose.....	Monosacch.	Cigar-shaped bac., light blue.	As at 15 minutes.....	As at 15 minutes.....	—
Arabinose.....	Monosacch.	Negative.	Negative.	Negative.	—
Xylose.....	Monosacch.	Negative.	Negative.	Negative.	—
Rhamnose.....	Monosacch.	Negative.	Negative.	Negative.	—
Glucose (Dextrose).....	Monosacch.	Pseudo-yeasts: Dark brown and swollen.			
		Cigar. bac.: Dark blue bands.	As at 15 minutes.....	As at 15 minutes.....	
		Bacteria: Blue.....			
		As for glucose, but more blue bacteria.	As at 15 minutes.....	Many more blue bact.	
Laevulose (Fructose)....	Monosacch.	Negative.	As at 15 minutes.....	As at 45 minutes, but numerous blue bac.	
Lactose.....	Disacch.	As for glucose.....	Negative.	Cigar. bac.: Blue.....	
Maltose.....	Disacch.	As for glucose, only	As at 15 minutes.....	As at 45 minutes.....	
Saccharose.....	Disacch.	Pseudo-yeasts only showing brown patches	As at 15 minutes.....	Pseudo-yeasts: Dark brown and swollen.	
		Negative.	Some small bac. brown	Fairly numerous brown and blue bact.	
Cellulose.....	Disacch.	Pseudo-yeasts: Light brown.	Deeper brown.....		
Raffinose.....	Trisacch.	Cigar. bac.: Blue bands	Deep blue.....	As at 15 minutes.....	
		Bact.: Some are light blue.	Light blue.....		
Mannite.....	Alcohol.	Pseudo-yeasts: Brown poles.	Brown extending from poles.	Dark brown.....	
		Cigar. bac.: Blue bands	Blue throughout.....	Dark blue.....	
		Bact.: Light blue....	Light blue.....	Blue.....	

TABLE 3 (continued).

Substrate.	Nature of Substrate.	PERIOD OF INCUBATION AND REACTION OF ORGANISMS.			
		15 minutes.	45 minutes.	3 hours.	20 hours.
Sorbite.....	Alcohol.	Pseudo-yeasts: Light brown. Cigar. bac.: Blue bands Bact.: Negative..... Negative. Pseudo-yeasts: Brown poles. Cigar. bac.: Blue..... Bact.: Negative.....	As at 15 minutes..... Negative. Negative. Brown..... Dark blue..... Small No. blue.....	As at 15 minutes..... Blue..... Blue bact.: Rare..... Negative. Negative. As at 45 minutes.....	As at 15 minutes..... Blue..... Blue bact.: Rare..... Negative. Negative. As at 45 minutes.....
Isodulcite..... Inosite..... Salicin.....	Alcohol. Benzol. Glucoside.	Pseudo-yeasts: Brown poles. Cigar. bac.: Blue..... Bact.: Negative.....	As at 15 minutes..... Negative. Negative. Brown..... Dark blue..... Small No. blue.....	As at 15 minutes..... Negative. Negative. As at 45 minutes.....	As at 15 minutes..... Negative. Negative. As at 45 minutes.....
Inulin.....	Polysacch.	Pseudo-yeasts: Brown spots. Cigar. bac.: Light blue. Bact.: Negative..... Negative.	Spots larger..... Light blue..... Negative.....	Brown spots and poles. Blue..... Small No. blue..... Negative.	Brown. Blue. Fair No. blue. Pseudo-yeasts: Some are brown. Cigar. bac.: Negative. Bact.: Fairly numerous blue ones. Pseudo-yeasts: Some brown. Cigar. bac.: Negative. Bact.: Fair No. blue.
Pectin.....	Polysacch.	Negative.	Negative.	Negative.	Negative.
Untreated Maize Starch..	Polysacch.	Negative.	Negative.	Negative.	Negative.
Soluble Starch.....	Polysacch.	Negative.	Pseudo-yeasts: Light brown. Cigar. bac.: Negative. Bact.: Fairly numerous blue.	As at 45 minutes.....	As at 3 hours.

ISOLATION OF AN IODOPHILIC STREPTOCOCCUS AND SOME OF ITS CHARACTERISTICS.

A small coccus was observed to be the dominant iodophilic bacterium, always closely associated with starch granules in the rumen and present in every sheep examined. An attempt was, therefore, made to isolate this organism and study some of its characteristics in pure culture.

A small silk bag of natural silk as described by Quin, v.d. Wath and Myburgh (1938), was filled with crushed and shelled sterilized maize kernels. This was suspended in the rumen through the fistula. After 48 hours of ruminal digestion the bag was withdrawn and transferred into a sterile petri-dish. It was then opened and a few partly digested kernels dropped into sterile saline. From this suspension surface cultures were made on dextrose-agar or starch-agar slants and incubated at 39° C. for 24 hours. Two or three types of colonies were usually present after incubation. Of these, the smallest dewdrop type of colony almost invariably proved to be the streptococcus associated with the maize kernels in great numbers. Subcultures were made on dextrose-agar. Smears were usually made of some of the kernels and stained with Lugol's iodine and with Gram's stain. Cluster of Gram positive and iodophilic cocci were observed in most instances around the starch particles (Photo 4, Plate I). Although some other organisms were usually also present, they occurred only in small numbers and were not iodophilic. In a similar way, chemically pure cellulose suspended in a separate compartment simultaneously with maize, was found to encourage various types of *Gram negative* bacteria (Photo 3, Plate III). A few Gram positive bacilli, coccobacilli and large cocci were also present. Casein (79 per cent. protein) *encourages predominantly Gram positive organisms*, comprised also of large cocci, cocco-bacilli and bacilli (Photo 2, Plate III).

The indications are, therefore, that materials submitted to digestion in the rumen encourage specific types of bacteria to multiply in its immediate surroundings. In the case of starch the organism concerned was found to play a significant role in its disintegration within the rumen. The organisms associated with the cellulose and casein probably also assist in their disintegration.

Some Characteristics of Isolated Streptococcus.

1. Size: .65 microns (First subculture).
2. Strongly Gram positive.
3. Non-sporulating ovoid cocci usually in pairs or short chains.
4. Growth:—
 - (1) Grows easily in dextrose broth with final pH=4.0.
 - (2) Dextrose- and starch-agar—in 24 hours at 37° C. discrete semi-transparent colonies.
 - (3) Does not grow in peptone water pH=7.5.
 - (4) Serum agar—chains up to 10 organisms. Mostly appears in pairs, longitudinally, or in chains of 4 to 5.
 - (5) Serum broth—short chains of 4 to 5. Longer chains of 12 to 15 infrequent.
 - (6) No liquefaction of gelatin.

- (7) Good growth in gelatin at room temperature (25° C.).
- (8) Methylene blue in milk not reduced.
- (9) Acid formed in milk, but no coagulation.
- (10) Growth on ordinary agar feeble. More profuse on dextrose-agar, soluble starch-agar and serum-agar. No growth on dextrose-agar, at room temperature in 24 hours, but minute colonies are present after 48 hours.
- (11) Broth—Uniform granular turbidity in broth after 24 hours and even earlier. Heavy deposit. Chains short.
- (12) Starch—In synthetic medium (Woodman & Stewart, 1928), the organism ferments starch with the production of acid, but no gas.
- (13) After three months in stabculture (dextrose-agar) sealed with and kept with wax at room temperature, growth was produced on dextrose-agar at 37° C. within 24 hours.

5. Fermentation of Sugars:—

- (1) Ferments: Glucose, Lactose, Saccharose, Maltose, Laevulose, Raffinose, Arabinose and Inulin to produce acid but no gas.
- (2) Does not ferment: Mannite, Galactose, Salicin, Sorbite, Rhamnose, Inosite and Dulcitate.

It also ferments *Aesculin* in bile medium producing acid.

6. Resistance to heat:—

Withstands heating in broth at 60° C. for 20 minutes, but is killed after 30 minutes.

7. Pathogenicity for laboratory animals:—

Fresh (24 hour old) broth cultures were found non-pathogenic to rabbits and mice on intravenous and intraperitoneal injections of .5 c.c., 1 c.c. and 1.5 c.c.

SUMMARY.

1. The only diastases present in the rumen seem to be those secreted by ruminal bacteria, which would then be responsible for the entire starch disintegration seen there.

2. In the rumen of sheep receiving a regular supply of starch in the diet, the bacterial disintegration of starch granules commenced after 5 hours and was completed within 18-20 hours. In the case of sheep not receiving starch in the diet, disintegration of the starch administered through the fistula commenced after 7 hours and took 8-10 hours to complete.

3. Starch granules of the various cereals differ in size and shape. The diameter of starch granules influences their rate of disintegration within the rumen.

4. Some of the products of starch degradation are resynthesised into glycogen and starch-like polysaccharides within certain ruminal bacteria and pseudo-yeasts.

5. The iodophilic micro-organism encountered in the rumen and associated with the disintegration and digestion of starch, are described.

6. An iodophilic streptococcus closely associated with the disintegration of starch was isolated in pure culture and some of its characteristics described.

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PLATE 1. BACTERIAL DIGESTION OF STARCH.

Photo 1.—Potato Starch Granules $\times 220$.

Photo 2.—One hour after administration of starch into rumen a few iodophilic cocci are seen attacking it.

Photo 3.—After 2 hours the iodophilic organisms have increased.

Photo 4.—After 4 hours a cluster of iodophilic bacteria are surrounding the starch granule.

Photo 5.—After 8 hours excavation of the surface of a starch granule is well advanced.

Photo 6.—26 hours only remnants of a granule are left. They are surrounded by iodophilic bacteria.

PLATE 2. RUMINAL IODOPHILIC MICRO-ORGANISMS $\times 500$.

Photo 1.—Pure culture of Gram Positive Iodophilic Streptococcus.

Photo 2.—Iodophilic streptococcus (large type).

Photo 3.—(a) Iodophilic streptococci photographed in the last stages of their iodophilic reaction and grouped around the remnants of a starch granule.

(b) Iodophilic tetracoccus.

(c) Small iodophilic bacillus.

Photo 4.—Showing numerous pseudo-yeast cells filled with glycogen. Several are showing a central constriction and partition in the act of dividing.

Photo 5.—(a) Large cigar-shaped bacilli. These bacilli represent various stages of starch synthesis within them. Some have blue discs, others blue rings and those farther advanced have broad dark blue confluent discs.

PLATE 3. INTRARUMINAL DIGESTION OF SPECIFIC FOOD-SUBSTANCES $\times 1,000$.

Photo 1.—A starch granule surrounded by an almost pure culture of the gram positive streptococcus.

Photo 2.—Casein taken from a silk bag suspended in the rumen for 36 hours. A rather mixed flora is seen, consisting mainly of large Gram positive cocci, coccobacilli and bacilli. A small number of Gram negative organisms are present.

Photo 3.—Chemically pure cellulose suspended in the rumen for 36 hours. A predominantly Gram negative flora is present. Relatively few Gram positive organisms are present.

PLATE I.

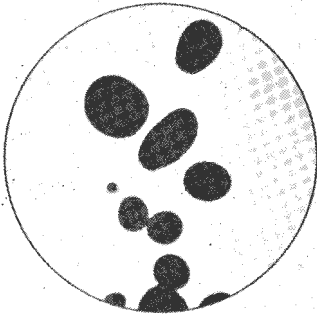


FIG. 1.

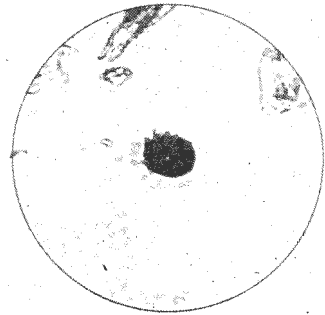


FIG. 2.

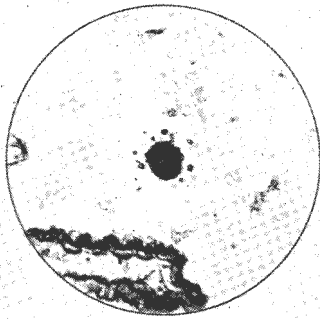


FIG. 3.

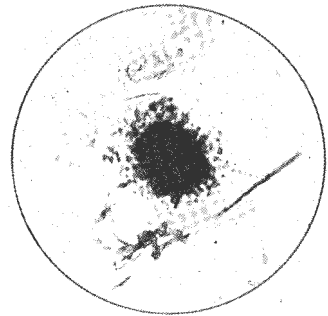


FIG. 4.

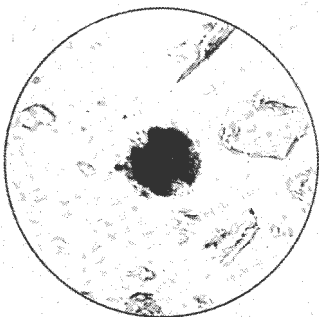


FIG. 5.

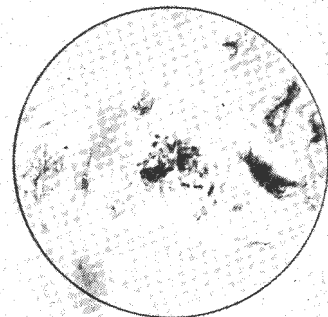


FIG. 6.

PLATE 2.



FIG. 1.

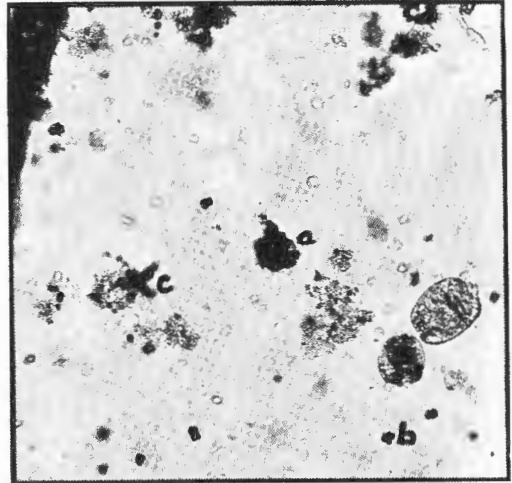


FIG. 3.

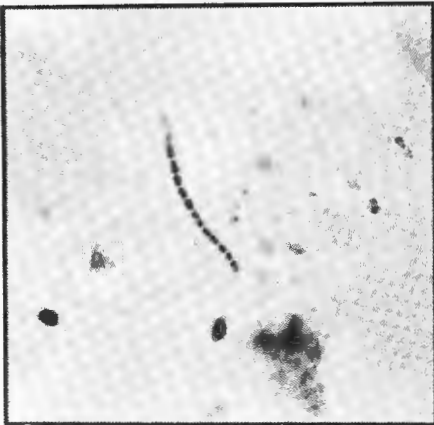


FIG. 2.

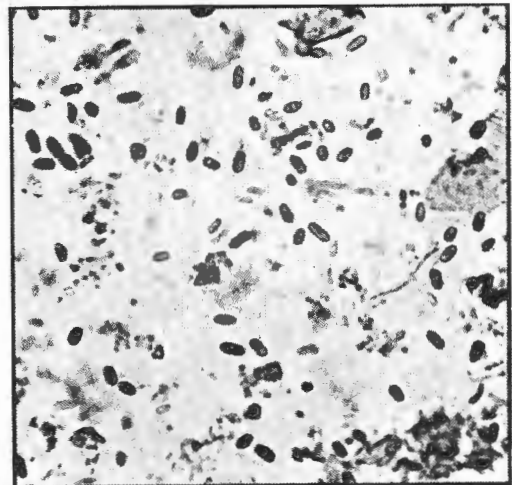


FIG. 4.

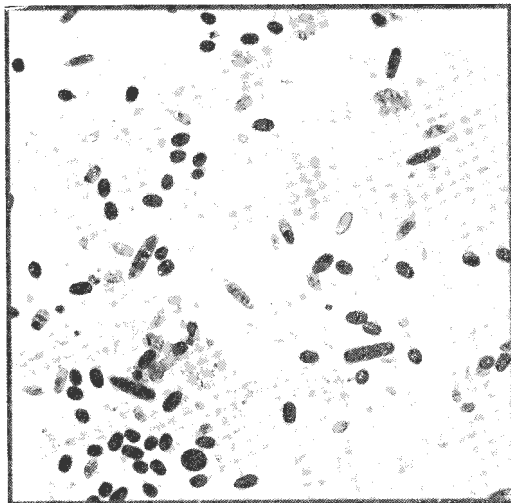


FIG. 5.

PLATE 3.

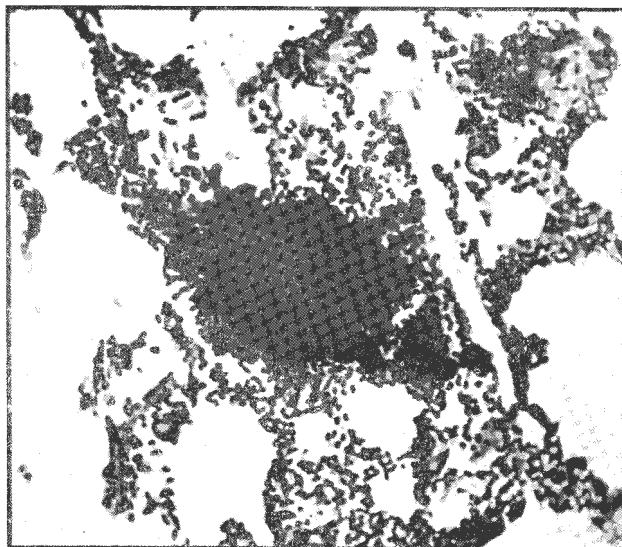


FIG. 1.

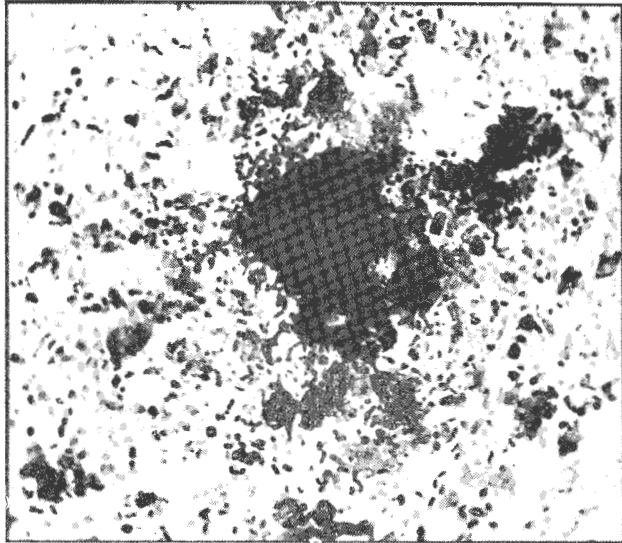


FIG. 2.

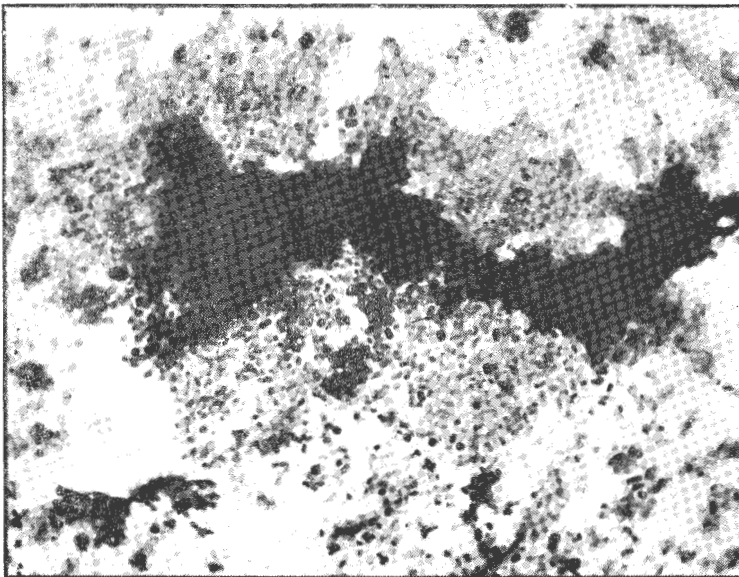


FIG. 3.