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The Influence of the Ration Upon the Intestinal Flora of Swine

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SUMMARY.

1. There is great individual variation in the mixed intestinal flora of pigs.

2. There is great individual variation in so far as the colon bacilli are concerned, as shown by the biometrical method of arranging its characters upon carbohydrates.

3. This individual variation masks any variation that may exist between groups of pigs fed on corn alone as compared to pigs fed on corn and protein.

4. There is a tendency for certain saprophytic types (spirilla, *fluorescens liquefaciens,* molds, etc.) to disappear when pigs are fed on corn alone.

5. There is a tendency for a diet of corn alone to throw the varieties of the colon bacillis into the *B. communis* variety rather than into the *B. communior* variety. This is not due entirely to the physical condition of the feces, as the poverty of *B. communior* was as marked in the contents of the intestine as in the feces.

6. Small variations in diet affect the intestinal flora, but very little as compared to highly carbonaceous or nitrogenous diet.

7. A study of the fermenting capacity of the colon bacilli will not explain the difference in metabolism in pigs on a strict corn diet, and one of corn plus milk albumen.

8. It is not possible to attribute the stunting effect of a strictly corn diet to marked variation in the bacterial flora of the alimentary canal as determined by the present technique.

Acknowledgments are due Pres. H. J. Waters, Dean J. T. Willard and Prof. W. A. Cochel for cooperation and suggestions; and to Prof. C. M. Vestal for weights and photographs of the pigs.

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The Influence of the Ration Upon the Intestinal Flora of Swine.*

L. D. BUSHNELL and J. J. FREY.

INTRODUCTION.

EXPERIMENTS in animal nutrition, particularly those of Waters, have shown that swine fed with corn alone develop very poorly, while swine fed on corn plus a very small amount of such a nitrogenous substance as milk albumen develop normally. It would seem that such differences in ration and metabolism must influence the intestinal flora, hence the problem of determining these changes and their possible influence was undertaken.

BRIEF HISTORICAL REVIEW OF LITERATURE.

The question has often been raised as to the significance of the microorganisms in the alimentary canal—are they essential to the perfect assimilation of food, or should they be regarded as purely parasitic?

RELATION OF BACTERIA TO THE DEVELOPMENT OF HIGHER ANIMALS. Duclaux (20), as early as 1885, showed by simple experiment that a bacterial association was necessary for the assimilation of more complex forms of nitrogen by higher plants.

In a discussion of the results secured by Duclaux for plants, Pasteur (56) made the following statement: "Often in our conversation in the laboratory, for many years, I have spoken to the young scientists of the importance there is in feeding the young animal (rabbit, guinea pig, dog and chicken) from birth, with pure nutritive material. For this last statement, I intended to designate food products which we would deprive completely and artificially of common microbes. . . . With no desire to affirm, I do not conceal that I undertake this study, if I have time, with the preconceived idea that life in these conditions becomes impossible."

* A part of the data in this bulletin was used as a thesis at the University of Kansas by L. D. Bushnell 1915, and part as a thesis at the Kansas State Agricultural College by J. J. Frey in 1916.

Although the above created a great deal of discussion and the institution of several elaborate experiments for its proof, the exact significance of the bacteria in the alimentary canal has never been definitely established. Some authorities, particularly Metchnikoff and his school, consider that the microorganisms which inhabit the intestinal tract produce all sorts of toxic substances which cause acute chronic intoxication of the organism. This terminates in symptoms which characterize old age. Others believe that these microorganisms are merely saprophytic forms which, through long association, have become adjusted to the conditions in the digestive tract where they exert no influence upon the host except occasionally when they assume pathogenic properties. Still others consider the intestinal bacteria as valuable in overcoming invaders, which if allowed to develop unchecked might seriously affect the host, and also in crowding out the putrefactive types which produce the toxic decomposition products, particularly from nitrogenous foods. Some authorities go so far as to affirm that the normal intestinal bacteria are essential for proper digestion, especially in higher mammals (Herbivora).

The first attempt to establish this relationship was by Nuttall and Thierfelder (55) upon guinea pigs. The young animals were removed, just before normal birth, by Caesarian section and placed at once in sterile cages. The animals were kept for eight days under absolutely aseptic conditions and fed on sterile crackers and milk and water. Under this treatment the animals increased normally in weight. Of the four animals carried through the experiment, the increase in weight was 5.5, 14.0, 16.0, 28 grams, respectively. This experiment is especially valuable, since guinea pigs, unlike most related animals, come into the world in such a state of development that they can properly assimilate the food of the adults, and these animals are especially likely to become infected with bacteria from their food. On the other hand, this experiment shows that guinea pigs can subsist upon crackers and milk in the absence of intestinal bacteria; but it does not necessarily follow that the same result would have been obtained if food rich in cellulose had been given.

According to the criticism of this work by Schottelius, it was possible to account for the increased weight of these animals by the amount of undigested food in the alimentary

canal. Nuttall and Thierfelder, upon examining the pigs at the conclusion of their experiment, found that the colon and cæcum were distended with brown caseous material. Schottelius was able to keep new-born guinea pigs alive for ten days by giving them nothing but sterile water. He also showed that the intestinal contents of a normal ten-day-old guinea weighed from twelve to fifteen grams, and in this case the intestine was not completely filled.

Schottelius (66) attempted to solve this problem by the use of chickens that were hatched and kept under sterile conditions. These chickens were fed upon sterile food and water for seventeen days. Although the chicks ate abundantly, they showed continuous hunger and declined about as rapidly as starved birds. In fact, some of the birds were moribund at the end of the second week. When *B. coli* from the feces of the hens was mixed with the food, they developed normally and increased in weight; in fact, more rapidly than check birds allowed to run free in the laboratory. This author has proved to his own satisfaction, at least, that intestinal bacteria, and especially the *B. coli* type, are essential to the normal development of chicks, and probably of other animals as well. He summarizes what he considers to be the manner in which normal bacteria influence the host:

(1) The intestinal bacteria are necessary for the nourishing of vertebrate animals and man.

(2) The benefits of the normal intestinal bacteria consist *(a)* in the preparation of the ingesta for the resorption of the nourishment; *(b)* in the irritation of the intestinal wall, thus increasing peristalsis; *(c)* in the luxuriant growth and the destruction of the pathogenic bacteria which get into the intestine; and *(d)* in the strengthening of the body against the pathogenic bacteria and against bacterial poisons.

Insects have been employed in working out this problem. Delcourt and Gueyenot (18) succeeded in keeping flies of the genus *Drosophila* for as many as twenty generations under aseptic conditions, finding that growth in sterile cages was perfectly normal and fully as rapid as in similar but contaminated cages. It also developed that the mortality was remarkably reduced, almost all the eggs from the sterile flies hatched, while in the septic cages whole broods would frequently die.

Bogdanow (3) and Woolman (76) worked with flies *Lucilia cæsar* and *Calliphora vomitoria.* Bogdanow found that while the larvae would develop under aseptic conditions, their growth was greatly retarded.

Woolman was able to carry his larvae through from sterile eggs to normal adults. During the first few days after hatching the growth of the sterile maggots was noticeably slower than those in septic cages. This temporary retardation (due to high temperatures in the sterilization of media) was soon overcome, and those kept sterile developed at the same rate as the checks. Maggots which were hatched from sterile eggs and treated with cultures of *B. coli, B. proteus vulgaris* and *B. putrificus* showed no more rapid development than those not so treated. In fact, those receiving *B. putrificus* died regularly before reaching the pupa stage.

Portier (58), in studying the larvae of certain leaf-mining microlepidoptera, found 30 percent of the *Lithocolletis* larvae were wholly free from bacteria, while 100 percent of the larvae of the species of *Nepticula* infesting the rose, which did not void their excrement on the interior of the mine, were also sterile.

Cohendy (14), in a more recent work upon .this problem, has apparently proved that life, in the case of chickens, is not only possible, but that chicks hatched and reared under aseptic conditions were more resistant to unfavorable environment than those harboring bacteria in the digestive canal. He was able to keep his chicks under aseptic conditions for forty days with no difference in growth between the sterile and the check birds. When the birds which had been sterile were placed under normal conditions, they developed into normal adult birds. He concludes as follows: "Life without microbes is possible for a vertebrate, the chicken, normally provided with a rich microbial flora. This aseptic life involves no deterioration of the organism."

Mme. O. Metchnikoff (50) and Moro (53) carried out experiments with tadpoles and larvae of the mud frog, Mme. Metchnikoff working with *Rana temporaria* and Moro with *Pelobates fuscus.* These authors concluded that there is some relation between the intestinal bacteria and the digestive function. In the work of Metchnikoff the mortality rate was higher among the nonsterile than among the sterile tadpoles. Al-

though the sterile ones lived longest, their increase in weight and length was much slower than the normal nonsterile checks. Moro was able to keep his tadpoles sterile for thirty-six days. It was found, however, that when the sterile tadpoles were placed in the water containing the feces of the mother, the increase and general development was much more rapid than in the case of those kept sterile.

Levin (47) makes the interesting statement that the aliamentary tract of polar animals is for the most part sterile. He examined arctic animals in Spitzbergen. The digestive tract was found to be almost entirely sterile in white bears, seals, reindeer, eider ducks, penguins, etc., although a very small number of organisms resembling the colon bacillus were found in one white bear and in two seals which were examined. He accounts for this because of the rarity of bacteria in the air and water. (It was estimated that there was but one organism in 11 cc. of water.) These animals were in a state of robust health, thus proving that intestinal bacteria were not essential to their normal metabolism.

When these animals are brought into temperate regions, however, they develop an active intestinal flora without serious injury to themselves.

Metchnikoff and others (51) studied the intestinal flora of the frugiverous bat, known as the "flying fox," imported from India. There were almost no bacteria displayed in preparations made from these animals. They were found only at certain points in particles of undigested fruit, which comprises the nourishment of the bats. At the same time no odor of putrefaction was observed; only the odor of fruit upon which the animals were fed.

Mlle. Tsiklinsky (74) recently published results of her investigations upon the intestinal flora of the insectivorous bat. The results obtained with these animals were analogous to those of Metchnikoff and his collaborators.

The reason for the poverty of bacterial flora in these animals is given by Metchnikoff as being due to the structure of the alimentary canal. These animals have an underdeveloped large intestine; that is, it is very short and no larger than the small intestine. The cæcum is entirely absent. The passage of the food is very rapid, defecation taking place in one and one-half to two hours after the absorption of the nourish-

ment. Thus the conditions are not favorable for bacterial development, as the intestine is freed from bacteria more or less mechanically. The reason given for the comparative longevity of these animals is because of the freedom of its intestinal tract from bacteria. Mlle. Tsiklinsky concludes her article with the statement that the absence of bacteria in the intestine causes no trouble in the life of highly developed beings, as the mammals.

The weight of evidence, therefore, seems to be against Schottelius, Bogdanow and others who have held the view that life without bacteria is impossible.

However, the conclusion can not be drawn that bacteria play no part in the physiological economy of animals, as it is a well-known fact that certain exogenous pathogenic microorganisms may be carried into the digestive tract with food and water. Here they may develop and produce most profound physiological changes in the host. The subtler influences of the normal intestinal flora are not so well established, but definite evidence of bacterial action in the digestive tract is not wanting.

Armsby and Fries (2), in studying the influence of fermentation on energy metabolism, estimate the production of about 4.5 grams of CH_4 per 100 grams of digested carbohydrates.

Von der Heide, Klein and Zuntz (78) (cited by Armsby and Fries) computed from Markoff's experiments that methane fermentation in cattle gives rise to the evolution of 4.374 calories of heat per cc. of methane, equivalent to 6.07 calories per gram. Roughly, from 9 to 17 percent of the increase in metabolism has its source in the methane fermentation.

This varies to some extent with the individual, is higher on concentrates than on coarse fodders, is higher with timothy hay than red clover and alfalfa hay and is greater on light than heavy rations.

This is probably due largely to the action of bacteria upon the food in the digestive tract.

HARMFUL INFLUENCE OF BACTERIA IN THE INTESTINAL TRACT. Metchnikoff, in his essays in 1903 and 1907, stated that toxic products from the intestine are responsible for symptoms of old age. Various products of bacterial action are known, to be toxic and give rise to intoxication of the body.

The origin of this intoxication called "autointoxication" is discussed by Wells (75) as follows:

"The sources of the poisons arising from the gastro-intestinal tract are numerous. They may be formed either from the food-stuffs, or from secretions and excretions of the body that enter the alimentary canal; and they may be formed either by the digestive ferments or by the bacteria, of the intestinal contents. Hence the number of these products is enormous, and we are by no means sure that those that have yet been identified include the most important or the most toxic."

This author gives a classification based upon the sources of the substance.

Indol formation is usually selected to indicate bacterial action in the intestine. This was definitely proved by Herter (30), who states that indol is not formed during tryptic digestion of proteids, and probably can not be formed in the course of physiological processes without the intervention of bacteria. This author studied the effect of introducing large numbers of *B. coli communis, B. proteus vulgaris* (Hauser) and *B. acidi lactici* into the intestinal tract of dogs, with a view to determining the effect upon indol production. It was found that the injection of pure cultures of colon bacilli into the jejunum of dogs was followed by an increase in the indican in the urine. This amount could not be accounted for by the slight amount injected with the culture. Similar experiments with *B. proteus vulgaris* gave negative or only slight effects upon excretion of indican. The other type of bacteria used tended to decrease the indican in the urine.

Rougentzoff (65) in a recent series of experiments made upon starving rabbits, as compared to those fed upon carrots, noted that increased inanition was followed by increase of colon bacilli in the alimentary canal and an increase in indican in the urine up to a certain point. He concludes that the researches permit him to suppose that the indican in starved animals is of intestinal origin. Perhaps by the action of *B. coli* upon the digestive juices, which are rich in albumen, more indican is produced in the case of starved rabbits than in case of those fed upon carrots, because the presence of sugar in the carrot-fed animal prevented *B. coli* from producing indol from the nitrogenous products present.

Berthelot (8) and Berthelot and Bertrand (9) have recently made important studies upon the production of toxic products by intestinal bacteria and their significance in the production

of autointoxication. These authors have found three organisms capable of producing *B. imidazoléthylamine* from histidin. This base shows characteristic physiological properties when injected. The chief organism isolated was termed *B. aminophilus intestinalis.*

BENEFICIAL INFLUENCE OF BACTERIA IN THE INTESTINAL TRACT. There are authorities who consider that bacteria, at least those normally inhabiting the alimentary canal, are indirectly beneficial to the organism. Thus the real significance probably does not lie so much in any immediate relation to digestion, but to the fact that the types whichare able to adapt themselves to intestinal conditions *(e. g., B.. coli* group; *B. lactis ærogenes; B. bifidus,* the bulgarian bacillus group, etc.) discourage the occasional invader which might otherwise be harmful to the host. Herter believes that the chief significance of the obligate intestinal bacteria lies in their potential capacity for thus checking the development of other types of organisms. This author considers that the proteolytic enzymes destroy bacteria low in vitality. The acid in the gastric juice probably has some bactericidal action. The exact nature of the action of the "obligate" upon the "wild" types is not well understood.

Metchnikoff in his studies with the cholera vibrio found that certain microbes had power to prevent its development, while others, on the contrary, favored the appearance of infection.

The researches of Bienstock (10) upon the factors which retard putrefaction are of interest, as he observed that *B. coli* and *B. lactis ærogenes* prevented the action of *B. putrificus* upon albuminoid substances. A well-known illustration of the inhibiting action of one group of organisms upon another is the case of the lactic acid bacteria which prevent putrefaction of raw milk.

According to Eijkmann (22), Conradi and Kurpjuweit (15), organisms of the colon group produce germicidal substances both thermostabile and thermolabile, active in dilutions of 1 to 100,000 parts. These "autotoxins" of Conradi and Kurpjuweit are considered to exert inhibitory action upon the organisms producing them, as was shown by the fact that the colon bacilli tend to die out toward the lower end of the intestine. It is also noteworthy that relatively few living representatives

of the *B. coli* class are present in the stools in cases of chronic constipation.

Eijkmann, and later Conradi and Kurpjuweit, considered exhaustion of old cultures as due to this substance, which could be destroyed at 60° C. and inactivated by filtering through porcelain. The last mentioned authors claim to have noted this toxic substance in the feces of healthy man.

Herter does not agree with these authors, and adds that the inhibitory action of colon bacilli is due to the exhaustion of the media, and in part to the production of acid, indol, phenol, or other putrefactive or fermentative substances.

According to Moro and Murath (54), similar retarding substances are present in the intestine of nurslings. The bactericidal action is exercised upon a large number of microbes, and the bactericidal substances act as natural safeguards. In the absence of definite proof, they concluded that *B. coli* was the true producer of the impeding substances. A short time afterward Rolly (64) sought to verify the discoveries of Eijkmann and Conradi and Kurpjuweit, but could find no proof of the existence of similar substances in cultures or in the feces.

Kohlbrugge (43), in his studies of the protective action of the intestinal wall against bacteria, noted a rapid "autosterilization" of the contents of the small intestine in man and higher animals generally. Normally the organisms present are attached to a particle of food, and as the intestine becomes empty the bacteria disappear, leaving the intestine practically sterile. He also considers the cæcum and vermiform appendix, instead of being useless vestigial organs, as of great importance as a culture source for the colon bacillus.

Why are the normal intestinal bacteria not killed by a similar action? Lembke (46), Kohlbrugge and others have suggested that there is a "pseudosymbiosis" established between the cells of the intestinal wall and the bacterial cells in the alimentary canal. This relationship is more marked in the large intestine than in the small and more marked in the adult than in children.

Kling (42) has recently carried out extensive experiments, using the microbial products of *B. bifidus*. This author thinks that it is only the acid produced by carbohydrate fermentation which acts as a germicidal agent. Also that the acid reaction of the feces is due to the fermentation of sugars which are not

absorbed. He thinks also that the acid production prevents the localization of pathogenic microbes in the alimentary canal.

INFLUENCE OF DIET UPON THE BACTERIA IN THE INTESTINAL TRACT. One of the most important problems in the study of intestinal bacteriology is that of the influence of feeding upon the intestinal flora. As we have seen, there are two groups of bacteria in the intestinal canal; one the so-called "obligate," the other the "wild" or "facultative" group.

Escherich (23) found in new-born children, as did Popoff (57) in new-born calves, and Rahner (59) in newly hatched chickens, an intestine always free from bacteria. Very soon after birth, however, microbes may be noted in the feces.

Tissier (72) has divided the period which elapses between the time of birth and the establishment of a permanent intestinal flora in a normal nursling into three phases: (1) the aseptic phase; (2) the phase of increasing infection; (3) the phase of gradual transformation, which leads up to the final permanent or milk phase. This author found a great predominance of *B. bifidus* (Tissier) in the feces of breast-fed children, whereas in bottle-fed infants there was a more variable flora. A mixed diet of milk and other foodstuffs caused a greater variation than did cow's milk alone. The conclusion was, therefore, that the diet, to a large extent, determines the character of the intestinal microöganisms.

Besides *B. bifidus,* an organism which resembles it very closely inmany respects, *B. acidiophilus* of Moro, was found to be a constant inhabitant of breast-fed infants. Rodella (62) and Cahn (13) made similar observations. Tissier, however, claims to have found this organism only in the feces of artificially nourished infants.

Bienstock (10) and Escherich (23) consider the colon forms to be favored by a milk diet, while the putrefactive forms predominate on a meat diet. If one divides the organisms into a facultative and an obligate group, the facultative group will be influenced by the nature of the food, but the obligate types will be influenced little except in regard to numbers.

Eberle (21) conducted a series of experiments upon children fed on sterilized cow's milk and arrived at the following conclusions: (1) The number of bacteria in the feces is enormous, and is independent of the number of bacteria present in

the food; (2) the number of cultivable bacteria is only 4.5 to 10.5 per cent of the total; (3) the estimates vary with the temperature at which the plates are kept and with the medium (gelatin or agar).

Jacobson (36) found that even such a comparatively mild change in diet as from their mother's milk to human milk completely altered the intestinal flora of young dogs. *B. bifidus* appeared in large numbers and replaced the normal bacteria to such a degree that the flora closely resembled that of breast-fed babies. This diet did not, however, agree with the dogs, for they rapidly lost weight.

Wuttrich and Freudenreich (77) studied the influence of diet upon two cows. Dry fodder gave a greater number of bacteria in the feces than grass fodder. When the hay diet was changed to one of sour potatoes there was a decrease in the number of organisms. The increase in the first case was due to the increase in all types, but more especially to those contained in the hay, although there was some increase in the colon bacilli in the feces.

Tappeiner (71) studied the digestion of cellulose in the intestinal canal. He fed hay to cattle and noted the fermentation in the first and middle stomachs and also in the small and large intestine. The most important fermentation of cellulose is in the first stomach, the next in the large intestine, and none in the small intestine. He sought for cellulose digesting enzymes, without success, by inoculating the intestinal juices into artificial media containing cellulose. As there was no digestion, he concluded that the digestion of cellulose in the alimentary canal was due to cellulose-digesting bacteria. This argument in favor of the importance of this function loses much of its force if it is true, as lately maintained by Bergman (7), that most of the cellulose eaten by Herbivora is provided with intracellular enzymes capable of decomposing cellulose.

Some recent work by Hunter and Bushnell (34) in connection with fermentation in silage has shown that the important fermentations are of bacterial nature and not due to respiration of plant cells. This will probably prove true in the intestine of the Herbivora.

Brotzu (11) fed dogs with sterile food and believed that he could decrease the bacterial content of the intestine. Sucks-

dorff (70) found a marked decrease in number during a period of three days. These findings are not in harmony with those of Stern (69), Eberle (21), Hammerl (26), Ballner (5) and Belonowsky (6). Stern found no reduction in the number of intestinal bacteria of a person who received sterilized food for three days.

Hammerl (26) does not agree with Brotzu in that there is a decrease in the intestinal flora of dogs fed on sterile food. Nor could he notice any appreciable change from a vegetable to a mixed diet. He noticed, however, that there was a decrease or disappearance of common saprophytes (molds, *B. fluorescens,* etc.) from the feces.

Belonowsky studied the effect of feeding sterilized corn and sterile corn and milk upon the intestinal flora of mice. After seven months there were the same numbers of bacteria in the feces as at the beginning.

Kendall (41) has even shown that the total abstinence from food for thirty-one days did not eliminate the bacteria from the lower intestine of man. Starvation did, however, reduce the bacterial species to two—*B. coli* and *B. mesentericus.*

Lembke (46), in a study of this problem, fed dogs bread, mixed food, flesh and fat. While other types of organisms were changed, he found *B. coli* always present. He found, however, that when a diet of bread was changed to one of meat the numbers of *B. coli* greatly diminished and other organisms appeared to predominate. Eventually the colon bacillus was restored to full prominence on a long-continued diet of meat. This author also fed some known microorganisms, and in some cases could cause a destruction of the usual intestinal flora, but not in others.

Jungano (39) studied the influence of a meat diet, and found that the intestinal flora undergoes a very pronounced change when the rat is gradually put on this diet. *B. coli* is present, whereas on the ordinary diet this organism is absent from the feces.

De Gasperi (17) studied the change in the predominating species in white rats fed on a diet of mixed grain and bread, and rats kept on a diet of raw beef liver and hashed horse meat. The change in diet caused a marked variation in the bacterial content of the feces. The rats did not thrive well on the meat diet. One rat on an exclusive meat diet died after a

month . (The colon bacillus was found in the heart blood.) This author states that there are predominating species for an exclusive vegetable or exclusive meat diet.

Rettger and Horton (61) compared the intestinal flora of white rats kept on an ordinary diet of dog bread, carrots, sunflower seed, meat and salt, with that of white rats fed on an experimental diet containing pure protein. The normal rats were fed carrots and small amounts of boiled beef twice a week. .

The experimental diet was as follows:

Edstin, gliadin, zein, casein or lactalbumen.....	18%
Protein-free milk	28
Starch	29
Lard	25

Some of the animals were fed milk-powder diet:

Milk powder	60%
Starch	12
Lard	28

In discussing their results these authors state that it was quite apparent from the beginning that the differences between the intestinal flora of rats receiving the special diet and those which were kept on ordinary food were not as marked as might be expected. This may seem the more surprising since the prepared diets were comparatively free from bacteria.

Appreciable differences in the character of the intestinal bacteria were, however, always noticeable. The feces of the rats which were given the diets containing pure protein exhibited a more simplified flora than those receiving the usual mixed food. This transformation was brought about, as a rule, within a period of three to four days after the change from one diet to another. Furthermore, the flora remained reasonably constant, depending apparently upon the diet, When the ordinary diet was changed to the special food, few types of bacteria were found to be present, either in examinations made directly of stained films or by the standard methods of cultivation in agar. An increase in the number of Gram-positive organisms from 35-50 to 85-100 percent was frequently observed. There were no appreciable differences in the results, in so far as the individual pure proteins were concerned, with the exception of those observed in the feeding of zein.

In the zein rat an organism was found to be present in large numbers which was rarely observed in any of the others, This organism was apparently of the proteus group, closely resembling *B. proteus vulgaris.* There was, furthermore, a more mixed flora in this rat. The authors suggest that this exceptional finding may be due to the peculiar solubility properties of zein, and consequently a certain degree of indigestibility of this protein.

The diets containing pure protein had little, if any, influence on the number of cultivable bacteria in the feces. The authors concluded by stating that no definite relationship could be established between bodily conditions (growth, vigor, etc.) and the intestinal flora of rats receiving special diets.

According to Hart, McCollum, Steenbock and Humphrey (27), an extended study of the bacterial flora of the feces of their animals (cattle) did not reveal the presence of types of organisms peculiar to any one lot. There was as much variation in this respect among individuals of a group as between groups themselves.

Hull and Rettger (33) state that they were unable to give a satisfactory explanation for the marked change which takes place in the intestinal flora of the white rat when the diet is changed from bread and lettuce to one containing milk or lactose. Milk undoubtedly owes this property to the lactose which constitutes such a large proportion of its ingredients. Lactose alone of all the seven different carbohydrates fed is responsible for the bacterial transformation in the intestine. These authors state that feeding mixed grain, particularly oats and corn, show a strong tendency to increase the number of acidophilus bacilli in the intestine, while a diet of bread and vegetables does not encourage such a flora, but has the same general influence of an ordinary protein diet.

Sittler (68) has shown that the flora of bottle-fed children can be made to resemble that of nursing infants by the feeding of carbohydrate foods which on hydrolysis readily yield dextrose, as, for example, lactose.

On the other hand, the feeding of carbohydrates which on hydrolysis yield levulose tends to change the flora of the breast-fed infant to the mixed flora of children receiving cow's milk. Cane sugar, although it contains dextrose, is objectionable on account of the levulose group which it holds. Accord-

ing to Sittler's view, the striking difference which is usually observed between the intestinal flora of bottle-fed children must be due largely to a difference in the digestibility of mother's milk and of cow's milk.

Herter and Kendall (31) were the firstto study the changes in the intestinal flora in response to various diets. They attempted to determine the nature and degree of substitution of the bacterial types due to change in the food from one highly nitrogenous to one highly carbonaceous, and back again. They state the following:

> "Out experiments on kittens and monkeys show that an abrupt change in diet from a dominantly protein (meat and eggs) to a milk and sugar diet is followed by an alternation of physiological condition in three distinct ways: *(a)* in the nature of the .intestinal flora; *(b)* in the putrefactive products of the feces and urine; *(c)* in the clinical conditions. . . . The chief characteristic of the bacterial change is the gradual but rapid substitution of an acidiophilic, nonproteolyzing type of flora for a strongly proteolyzing type. The chief feature of the putrefactive conditions in the intestine is the reduction of indol, skatol, phenol and bound hydrogen sulphide and a diminution in the indican and aromatic oxyacids in the urine. Clinically, the most striking feature of the change in diet is an improvement in spirits and activity, which may safely be considered as showing a marked improved sense of bodily and physical well-being."

Torrey (73) has recently investigated the fecal flora in typhoid fever and its reaction to various diets. This author concluded that on a high-calory diet the intestinal flora tends to become simplified in regard to the variety of bacterial types observed in direct Gram-stained smears from the feces and in cultures, and to become converted into a fermentative type in which the dominant organism was *B. acidophilus.* The degree of this transformation was dependent largely upon the type of flora which was present at the onset of the disease. When the flora showed a definite putrefactive tendency, the change did not extend farther than the elimination of the obligate putrefactive organisms and a moderate development of the aciduric types; while, with a more favorable initial flora, the change was so radical in type that the stools finally resembled those of the first few years of normal infancy in the dominance of the *Bacillus acidiophilus,* and even in the presence of the *Bacillus bifidus.* Such a flora is not an obligate fermentative one and under the conditions of feeding is not capable of forming indol. Observations on a few patients given a purely milk diet indi-

cated that this food alone does not bring about this transformation of the intestinal flora to such a degree as that which may follow a more liberal carbohydrate feeding.

The typhoid bacillus was isolated from stools of these patients on a high-calory diet less frequently than has been reported for other series of cases in which the feeding was less liberal.

He also found that milk alone did not influence the intestinal flora of typhoid patients to such a degree as did a high-calory diet. In one of his cases he found a distinctly fermentative flora at the beginning of the trial, but after four days of milk feeding, organisms of the *B. acidiophilus* type had decreased greatly in number and in eight days they were almost entirely replaced by colon and streptococcus types. The fecal flora has thus undergone a transformation to one partially proteolytic and indol-forming.

As there was a history of milk diet for this patient for two weeks before admission to the hospital, about three weeks was apparently required to bring about the marked change in the character of the flora. The conclusion has been drawn, therefore, that diet to a large extent determined the character of the intestinal microorganisms.

It has been suggested that adventitious bacteria, frequently in considerable numbers, undoubtedly reach the intestinal tract from time to time. If these organisms can adapt themselves to prevailing conditions they may establish themselves at a certain level. It is doubtless through this process that certain types of intestinal bacteria have their origin. This point should not be entirely forgotten in attempting to study the influence of the diet upon the intestinal flora. While the total number of bacteria fed apparently makes little difference, the types fed with different diets may be of great importance.

Hart, McCollum, Steenbock and Humphrey (27) state that the method of feeding had little influence upon the digestive process as shown by their experiments. Evidently a "habit of metabolism" was not so firmly established by long-continued use of certain food materials as to make it impossible to change suddenly the nature of the animal's ration.

THE MANNER IN WHICH FOOD SUBSTANCES MAY INFLUENCE THE BACTERIAL FLORA. Jago (37) states that flour retards

the fermentative action of certain yeasts upon sugar. This is thought to be due to soluble proteins.

Michaelis (52) has pointed out that foreign protein matter is under all circumstances a deadly poison for yeasts, and that it is rendered inocuous by the proteolytic enzymes present.

Baker and Hulton (4) recently confirmed the conclusions that flour inhibits the fermentation in a solution of sugar by brewers' yeasts.

Lange (45) found that bruised grain or bran or meal, or even aqueous extracts of them, had a poisonous effect on yeasts. He further found that different kinds of yeasts varied in susceptibility, distillers' yeasts most resistant, top brewers' less, bottom brewers' still less. The injurious substances could be removed by heating.

Later other authors, Henneberg (29), Hayduck (28), Seyffert (67), have found that the toxic effects are completely obviated by the addition of a small quantity of inorganic salts, lime salts being most effective, magnesia next.

Delbruck (19) has always looked upon the yeast from the standpoint of its powerful defensive agencies. By sending out its by-products it protects itself against all organisms to which these are poisonous. He concludes that zymase is not only a respiratory but a fighting enzyme. Also the proteolytic enzymes digest proteins which are poisonous to the yeast. The law seems to be that in the struggle for existence those organisms which specialize in the production of fighting enzymes would be strongest.

In some recent work Greenfield (25) showed that environment apparently changes the ability of the colon group to ferment carbohydrates. Of all types from surface waters, 72 percent were acid to methyl red and 28 percent alkaline. From manufactured ice, 75 percent were acid and 25 percent alkaline. In natural ice, 32 percent were acid and 68 percent alkaline. The types that are alkaline to methyl red are supposed to be of nonfecal origin, and seem to be more resistant to undesirable conditions than those from fecal origin. From all natural sources the *B. communior* predominated over *B. communis.*

There is considerable evidence to show that diet influences types of organisms in the digestive tract. This may be explained by the ability of organisms to accommodate themselves

to the physiological and chemical conditions which prevail there. Also that the intestinal flora changes definitely from infancy to adult life as the diet changes from that of milk to a mixed diet. The flora of the breast-fed infant differs from that of the artificially fed infant. Kendall has noted that *B. bifidus* tends to increase in numbers when lactose is fed, while *B. acidophilus* increases. if dextrose and maltose are substituted for the lactose.

Kendall also states that there are two important factors to consider in discussing the influence of diet upon the intestinal flora: (1) the substitution of types of organisms, which frequently follows a monotonous diet; and (2) a change in the metabolism of existing types of intestinal bacteria when dietary conditions are such that the intestinal medium at one or another level fluctuates in its content of utilizable carbohydrates and other nutrient substances.

In the light of the preceding investigations it would seem that a grain diet might influence the intestinal organisms in some way which could be demonstrated, as it is evident that similar organisms respond to or disappear under a certain environment.

PLAN OF INVESTIGATION.

1. Material was collected from pigs fed different diets.
2. A study was made of the influence of these diets upon the intestinal flora as shown by use of Gram's method of staining.
3. A study was made of the influence of these diets upon the total numbers of bacteria in the feces.
4. A study was made of the influence of these diets upon the various types of bacteria in the feces.
5. A special study was made of the influence of these diets upon the *B. coli* group of bacteria in the intestinal contents and feces.

DESCRIPTION OF PIGS.

The animals used in these studies were being fed in nutrition tests at this station under the supervision of Pres. H. J. Waters, the department of animal husbandry and the depart ment of chemistry.

The pigs numbered C1, C100, C1000, C1110 were fed entirely on corn meal (ground medium fine) moistened with

water at feeding time. C in the number indicates a corn alone ration. Pigs numbered CP1111 and CP1210 were fed on the same corn meal plus the albumen from 12 pounds of milk to each pound of corn meal. CP in the number indicates a corn ration supplemented with protein.

Pigs C1 and CP1111 were litter mates. Pigs C1000 and CP1210 were litter mates. Pig C100, was from a third litter. Pig C1110was an animal which had been kept from a previous year's work.

All the pigs were put on the test as soon as weaned and continued upon this diet for 200 days. At the time of these tests pig C1110 had been fed on corn alone for approximately twenty months.

Table I shows the individual and average weights of the corn and corn and protein pigs. Table II shows gains in weight by pig C1110.

TABLE I.—*Weight.*

DATE, 1914.	C1.	C100.	C1000.	Average.	CP1111.	CP1210.	Average.
June 26	25	35	31	30.6	25	34	29.8
July 6	30	41	34	35.0	30	37	33.5
July 16	29	44	38	37.0	38	41	39.5
July 26	34	46	43	41.0	42	50	46.0
August 5	37	51	46	44.6	40	56	48.0
August 15	39	57	48	48.0	60	69	64.5
August 25	42	61	55	52.6	73	80	76.5
September 4	41	70	51	54.0	82	90	86.0
September 14	47	71	54	57.3	97	104	100.5
September 24	58	75	52	61.6	102	116	109.0
October 4	53	80	56	63.0	116	127	121.5
October 14	52	83	59	64.6	128	147	134.5
October 24	51	88	53	64.0	156	159	157.5
November 3	58	92	55	68.3	164	167	165.5
November 13	55	94	56	68.3	180	188	184.5
November 23	59	97	56	70.6	194	185	189.5
December 3	60	100	62	74.0	215	223	219.0
December 13	64	102	58	74.6	283	245	239.0
December 23	65	102	59	75.3	250	248	249.0
January 2	67	104	60	77.0	256	261	258.5
January 12	72	105	60	79.0	265	271	268.0

TABLE II.—*Showing Gains in Pig C1110.*

DATE.	Lbs.	DATE.	Lbs.	DATE.	Lbs.	DATE.	Lbs.	DATE.	Lbs.	DATE.	Lbs.
7-28....	50	10-26....	111	1-24....	110	4-24..	83	7-23..	104	10-21..	178
8- 7....	50	11- 5....	113	2- 3....	103	5- 4..	80	8- 2..	111	10-31.	192
8-17....	50	11-15....	114	2-13....	103	5-14..	.....	8-12..	119	11-10..	208
8-27....	56	11-25....	115	2-23....	103	5-24..	85	8-22..	128	11-20.	218
9- 6....	60	12- 5....	124	3- 5....	102	6- 3..	82	9- 1..	134	11-30..	238
9-16....	64	12-15....	125	3-15....	80	6-13..	82	9-11..	150	12-10..	243
9-26....	70	12-25....	113	3-25....	89	6-23..	87	9-21..	154	12-20..	252
10-16....	73	1- 4....	120	4- 4....	88	7- 3..	92	10- 1..	161	1- 9..	261
10-16....	103	1-14....	115	4-14....	87	7-13..	96	10-11..	173	1-19.	266

A brief inspection of the tables and figures shows: first, that there is considerable individual variation, especially in the corn pigs; second, that the addition of a very small amount of milk albumen either stimulates growth directly or overcomes some retarding influence; third, that the pigs were not only able to maintain but actually to increase in weight upon this diet.

Table II shows the influence of a long-continued regime of feeding on corn alone. For thirteen months there were but slight gains in weight. This increase could probably all be accounted for by the increase in growth. At the end of this period there is a sudden increase in weight, probably due mostly to the laying on of fat. This phenomena has been noted several times in the experimental pens at this institution and also in agricultural practice before the feeding of balanced rations came widely into vogue. On the other hand, there have been several animals in the experimental pens that actually starved to death when fed all the corn they would eat.

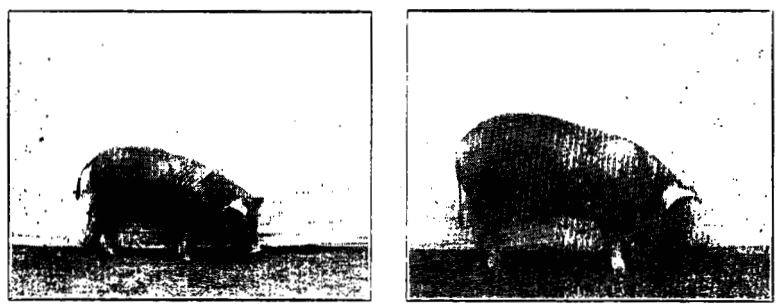
The photographs shown in figures Nos. 1 and 2, plate I, were taken at the same distance from the animals and with the same camera. A comparison of figures 1B and 2B, taken at the close of the experiment, shows the comparative development of the two animals. These animals were kept in a yard with a cement floor, so that there was no chance for them to get food other than that supplied.

There is a noticeable effect of this diet upon the spirits of the animals. The pigs fed on corn alone always appeared hungry, although the corn was continually within reach. They were uneasy and subject to troubles indicating malnutrition,

such as lameness, etc, They were usually somewhat constipated, but occasionally had diarrhea.

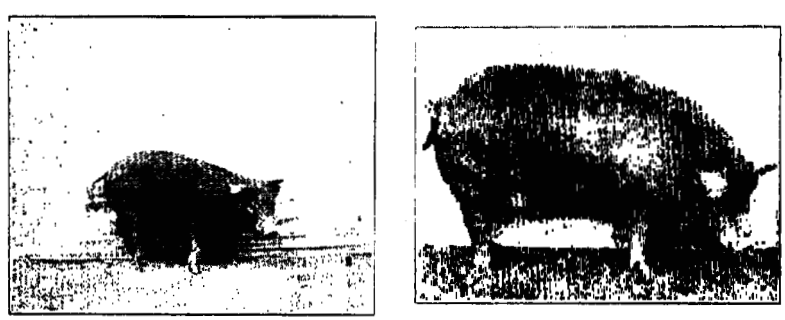
There was usually a marked difference in the physical condition of the feces. The feces from the pigs fed on corn and albumen were of a yellowish-white color, of rather soft consistency, though not fluid. The odor was sour rather than putrid. The feces of all the corn pigs were darker in color

PLATE I.



A. *B.*

FIG. 1. *A*, Pig C1 at the beginning of the feeding experiment. *B*, Pig C1 at the close of the experiment, 200 days on corn alone.



C. *D.*

FIG. 2. *C*, Pig CP1111 at beginning of the experiment. *D*, Pig CP1111 at the close of the experiment, 200 days on corn and milk albumen.

with a more putrid odor. The consistency was very firm, but on some occasions diarrhea was noted for a short time.

The pigs fed corn and albumen showed more undigested food particles than the corn pigs, but they also ate more. The feces of the corn pigs showed large amounts of straw, hair and other foreign material.

Pigs C1 and CP1111 were slaughtered at the close of the experiment, and an examination of viscera showed no indications of pathological change.

The problem was considered from two standpoints: (1) the influence of the diet upon the kind and number of organisms in the feces; (2) the change in the metabolic activities of certain existing types.

GENERAL METHODS.

COLLECTION OF MATERIAL. Material was usually collected from the rectum by means of a sterile glass spoon, or if enough could not be collected in this way a portion from the center of a freshly voided mass of feces was taken. This was placed in a sterile petri dish, taken directly to the laboratory and examined immediately. When material was taken from a slaughtered animal, portions of the intestine were tied off and removed to the laboratory intact.

MAKING THE SUSPENSIONS. For smears one gram of the fresh material was weighed out on a sterile paper and removed to a large test tube containing ten cc. sterile broth, emulsified by means of a stiff platinum loop, and then by shaking for three minutes. Smears and dilutions were made from this at once, but for colon isolations the material was allowed to stand at 37° C. for an hour to allow the clumps to settle and to permit a slight development of the bacteria.

MAKING SMEARS. The making of smears is an important bit of technique if they are to be used for quantitative work. Smears were made directly upon thoroughly cleaned slides by using a second slide held at an acute angle to the first. The same loop was used in all cases, and approximately equal amounts were transferred in each case. The smears were air dried and fixed in the flame.

STAINING SMEARS. Gram's method was used, as follows: Aniline gentian violet, 3 minutes; Gram's iodine solution, 3 minutes; rinse in water; decolorize in 95 percent alcohol, 3 minutes; rinse in water; counterstain with 1 percent pyronin, 1 minute.

The length of time the stain is applied is important, as some organisms appear Gram negative if treated for a shorter time. Also, a uniform technique should be followed to give comparable results.

MICROSCOPIC EXAMINATION OF SMEARS. Smears were examined for Gram positive and negative rods, and cocci, spirilla and spores. Quantitative estimations were made in some cases. In some cases the predominance of certain types was determined. It is very difficult to determine in all cases whether an organism is Gram positive or negative as seen in smears, as a part of the same organism may retain the stain and a part be decolorized.

The following table was obtained from an examination of smears made and stained as above described and examined very carefully. The figures are percentages of total numbers counted. This data was obtained by counting ten fields on each slide and a large number of slides from each animal. These determinations were made over a period of about three months.

TABLE III.

Pig.	Rods.		Cocci.		Spirilla.	Spores.
	Gram+.	Gram−.	Gram+.	Gram−.		
C1	6.9	73.0	1.5	16.5	.....	2.1
C100	13.6	66.5	4.5	12.7	.....	2.4
C1000	10.6	59.9	3.8	20.5	.....	5.1
C1110	11.9	66.3	2.3	14.9	.....	4.6
CP1111	14.4	52.5	4.9	21.8	0.9	5.5
CP1210	13.9	54.6	6 8	17.1	1.2	6.3

Table IV shows an average for the above. C1110 is not included in obtaining the average for the corn pigs, as this animal was from a separate lot.

TABLE IV.

Pig.	Rods.		Cocci.		Spirilla.	Spores.
	Gram+.	Gram−.	Gram+.	Gram−.		
C1 C100 C1000	10.3	66.5	3.3	16.6	.....	3.2
C1110	11.9	66.3	2.3	14.9	.....	4.6
CP1111 CP1210	14.1	53.5	5.8	19.4	1.1	5.9

This data is not suggestive, except perhaps in case of the spirilla. These organisms were not seen in smears from the feces of pigs fed on corn alone, One point of interest is the small number of spores in the corn-alone pigs. These animals

continually appeared hungry and ate a great deal of straw, bits of wood, hair, etc., substances that usually contain large numbers of spores. Apparently the saprophytic spore formers do not predominate as spores in the feces.

Table V was obtained from a second lot of animals later in the year. The data here given were obtained by examination of fifty-eight smears from the corn-only group and eighty-five smears from the corn-and-protein group, The corn-and-protein animals were fed on corn, shorts and tankage,

TABLE V.

Pig.	Gram+	Gram−.
Group C.....	10.9	89.1
Group CP.....	25.0	75.0

In this table the corn-and-protein pigs show more Gram-positive and fewer Gram-negative organisms than in case of pigs fed on less protein,

The latter might be explained by tables X and XI. In these tables the flora in the upper intestine was more dominantly Gram positive in the corn-and-protein than in the corn-alone pig. Thus probably more of this type would pass into the feces.

Table VI shows the influence of ash upon the intestinal flora as shown by Gram stain. The ash used was a modification of one used by Osborne and Mendel, and the protein was buttermilk casein.

TABLE VI.

Pig.	Gram+.	Gram−.
Group C without ash.....	22.9	77.1
Group C with ash.....	29.7	70.3
Group CP without ash.....	10.0	90.0
Group CP with ash.....	24.9	75 1

Table VII is an average of the two groups shown in table VI.

TABLE VII.

Pig.	Gram+.	Gram−.
Group C and CP without ash.....	16.4	83.5
Group C and CP with ash.....	27.3	72.7

These tables show little except that the ash increases the Gram-positive types in both the corn-alone and the corn-and-protein pigs; more in case of the latter than the former.

An interesting observation was made in studying these smears. It was repeatedly noted that in the corn-and-protein pigs the organisms did not stain as well as those from the corn-alone pigs. A more careful comparison showed that particularly the Gram-negative types in the corn-and-protein pigs took the stain more irregularly and many individuals stained very poorly, showing more or less disintegration. The tendency was three to fifteen times as marked in the corn and protein as in the corn-only pigs.

Herter and Kendall mention this in connection with the transitional flora as animals are changed from protein to a carbohydrate diet.

A comparison of the influence of the nutritive ratio shows the Gram-positive organisms predominate on a 1:3 diet; but this is reversed with a 1:4 diet, while a 1:6 diet showed the Gram-positive organisms as prominent as the Gram-negative.

On August 20 the pigs fed on a 1:3 ratio all showed a dominantly Gram-positive flora. The same animals on September 20 showed a reverse condition, but to a less marked degree. This shows that the Gram method is an index to the influence of diet upon the intestinal flora only within limits.

BACTERIA AT DIFFERENT LEVELS OF THE INTESTINE. Observations were made at different levels of the intestine upon two pigs which were slaughtered. Samples were taken from the stomach and at about each six-foot level to the rectum. The results are shown in tables VIII and IX.

TABLE VIII.—*Data from Pig C1.*

Level.	Gram stain.	Gas production.		Neutral red reduced.	*B. coli* isolated from Endo media.	Average number rods per field.		Average number cocci per field.	
		Dextrose.	Bile lactose.			G+	G−	G+	G−
Stomach.....	Dominant G−.. Few G+.....	0	0	+	+	2	5	0	0
Small intestine.	Dominant G−.. Many G+.....	0	0	+	+	5	7	0	0
Small intestine.	Types about equal.....	0	+	+	+	12	12	0	0
Small intestine.	Types about equal.....	+	+	+	+	19	19	0	0
Small intestine.	Dominant G−..	+	+	+	+	3	16	1	5
Small intestine.	Dominant G−.. Very few G+...	+	+	+	+	2	53	0	2
Small intestine.	Dominant G−.. Very few G+...	+	+	+	+	2	62	0	8
Small intestine.	Dominant G−.. Very few G−...	+	+	+	+	1	45	0	0
Ileocœcal valve	Dominant G−.. Very few G+..	+	+	+	+	2	113	0	5
Large intestine.	Dominant G−.. Few G− cocci..	+	+	+	+	9	295	0	20
Large intestine.	Nearly all G−.. Few Cocci.....	+	+	+	+	2	78	0	5
Rectum.....	Mostly G−.....	+	+	+	+	1	18	0	18

TABLE IX.—*Data from Pig CP1111.*

Level.	Gram stain.	Gas production.		Neutral red reduced.	*B. coli* isolated from Endo media.	Average number rods per field.		Average number cocci per field.	
		Dextrose.	Bill lactose.			G+	G−	G+	G−
Stomach.....	Dominant G+ rods.. Few organisms.....	0	0	0	0	3	0	0	0
Small intestine....	Dominant G+ rods..	0	0	0	0	9	2	0	0
Small intestine....	Dominant G+ rods..	0	0	0	0	2	1	1	0
Small intestine....	Gram+ and G− types about equal..	0	0	0	0	2	2	0	0
Small intestine....	Dominant G+.....	0	0	0	+	16	5	0	0
Small intestine....	Dominant G+.....	0	0	0	+	9	4	3	0
Small intestine....	Dominant G+.....	0	+	0	+	31	11	1	0
Ileocœcal valve....	Dominant G−..... Cocci present.....	+	+	+	+	11	90	4	7
Large intestine....	Dominant G−..... Cocci present.....	+	+	+	+	11	94	1	8
Large intestine....	Dominant G−..... Many cocci present..	+	+	+	+	35	134	12	24
Large intestine....	Dominant G−..... Many cocci present..	+	+	+	+	62	158	32	26
Rectum.....	Dominant G−..... Few cocci.....	+	+	+	+	3	26	2	0

These tables show that the Gram-positive types predominate to lower levels in the corn-and-protein than in the corn-alone pig. There is a marked change in the flora at the ileocæcal valve region in the CP1111 pig, much above that in the C1 pig, but in no case were there so many Gram-positive organisms as in the case of the CP1111 pig. Miscellaneous forms, mostly Gram-negative cocci, were observed more frequently in the corn-and-protein pig. The Gram-positive cocci were also about four times as prevalent in corn-and-protein as in corn-alone pigs.

All types of cocci grew more commonly from the feces of corn-and-protein animals than from the corn-alone animals. In a study of the predominating types isolated, a greater number were obtained from pig CP1111 at all levels except the stomach and the upper part of the small intestine. Fluorescent bacteria and molds from the CP1111 pig were also noted, but were not noted from corn-alone pig. This also holds true from a study of the fecal flora from the other animals of this group. *B. coli*-like organisms could be isolated from the stomach and at each level below to the rectum in the C1 pig, while the CP1111 pig showed no organisms of this sort from the stomach or for about eighteen feet below in the small intestine.

This appears to be important. Hess (32) has recently shown that breast-fed infants show fewer bacteria in the duodenal region than bottle-fed babies. Usually bottle-fed infants are not so well nourished as breast-fed infants and are more subject to troubles of a dietary nature. This may possibly be due to an excessive bacterial development in the region of the duodenum, with the resultant development and more rapid absorption of toxic products of bacterial decomposition. The corn-alone pig showed marked evidence of malnutrition, but with our present lack of knowledge it is impossible to say definitely whether this was due to chronic poisoning by bacterial action.

Another interesting point is that organisms of the *B. coli* group could be isolated from levels much higher in the intestine than those showing gas in dextrose or bile-lactose fermentation tubes. A careful study of these types showed them to be capable of fermenting both dextrose and lactose very vigorously.

There was practically no difference between the predominating varieties of *B. coli* in the upper intestine and in the

feces except in the case of pig C1. All the cultures (three in number) from the stomach were *B. communior.* In pig CP1111, 69 percent of the colon cultures from the intestine and 58 percent from the feces were *B. communior.*

TOTAL NUMBERS. The following counts were obtained by using equal amounts of freshly drawn blood mixed with the fresh fecal suspensions smeared upon slides, stained and counted directly. The counts were reduced to a dry basis.

From an average of twenty slides each from corn and corn-and-protein pigs 190,000,000 microorganisms per milligram were obtained for the corn-and-protein pigs and 174,000,000 per milligram for the corn-alone pigs.

The following counts were obtained from meat infusion agar plates incubated at 37° C. for forty-eight hours. The counts were reduced to milligram of dry feces. For the corn-and-protein pigs 42,000 per milligram, and for the corn-alone pigs 38,000 per milligram. From all the data obtained it was estimated that there are about 10 percent more organisms per gram in the feces of corn-and-protein pigs than in the feces of the corn-alone pigs. There was, however, great individual and daily variation.

In the case of two pigs slaughtered small numbers of organisms were found in the stomach and upper part of the small intestine. There is a gradual increase to the ileocecal valve region. Throughout the large intestine the number remained about the same, with a marked decrease in the rectum.

The number of organisms growing upon acid glucose agar (1 cc. of 1 percent acetic acid to 9 cc. of media) were about the same in each group.

Average number of aciduric types in fresh feces of corn and protein pigs, 757,000 per mg.; average number of aciduric types in fresh feces of corn pigs, 612,000 per mg. (Averages from 12 determinations. Plates were allowed to incubate five days at 37° C. before being counted.)

The number of proteolytic organisms was determined by the dilution method in gelatin incubated at 37° C. for 48 hours. Sixty times as many proteolytic types, on an average, were found in the feces of the corn and protein animals as in the feces of those having corn alone.

Average proteolytic types in fresh feces corn-and-protein pigs, 4700 per mg.; average proteolytic types in fresh feces

corn pigs, 78 per mg. (Average for 12 determinations. Gelatin tubes placed in ice box after 48 hours incubated 37° C. If they failed to solidify they were considered to have been liquefied by the organism.)

Average number of colon bacteria, 170 per mg. for CP pigs; average number of colon bacteria, 140 per mg. for C. pigs. (Determinations by dilution. in bile-lactose fermentation tubes.)

It is a well-known fact that all the organisms appearing on a stained slide are not living. Various estimates of the proportion of living organisms have been made from time to time, particularly in the case of man. According to MacNeal, Latzer and Kerr (49) the average for all determinations was 379,000,000 per milligram of fresh feces as determined by microscopic count. The average total numbers growing on all kinds of media used was about 50,000 per milligram, or approximately one of each thousand of those present was living. Eberle considered the number of living bacteria as only 4.5 per cent to 10.5 per cent of the total; other authors have made similar estimates at different times. In this work about one of each two hundred and fifty organisms would grow upon media.

When pigs are fed on corn alone, the nutritive ratio is about 1:9. This seems to be too wide for the best development of the protein-digesting types, but does not greatly influence the types which are able to ferment carbohydrates.

At first it was thought 'that the preceding data showed the influence of the diet, but the more the problem was studied the less possible it was to draw definite conclusions. Daily variations in a single individual were often contradictory, and duplicate animals showed opposite conditions in many instances hence it was impossible to explain why one group of animals developed so much better than a second group fed differently.

TYPES PRESENT—MISCELLANEOUS. As a part of the problem it was decided to study the influence of the diet upon a definite group of organisms. The predominating types isolated fall into five groups: (1) aciduric, (2) colon, (3) proteolytic, (4) comparatively inactive types, (5) anaerobes.

The aciduric organisms were apparently of one type-long; irregularly staining Gram-positive rods, arranged singly, and

in short chains. Growth on agar was almost invisible. The organisms did not liquefy gelatin or digest the casein of milk. They did not produce indol or reduce nitrates, but produced acid with no gas in dextrose. Diet seemed to influence their numbers to some extent.

The proteolytic types were studied quite in detail. These were obtained both from heated and unheated material. Three types predominated, but several others were isolated. These were not influenced by the method of feeding, except that they were somewhat more common from the corn-and-protein pigs. *B. proteus vulgaris* types were noted quite commonly from both groups of animals. There was a general variation of all types rather than of any one type.

There were more coccus types from the corn-and-protein pigs. Several types were isolated, but because of daily variation, and the fact that some of the types from stained smears were unrecognizable in cultures, it is impossible to discuss the significance of these types. Pig CP1111 developed many more than pig CP1210 fed in the same manner.

Great difficulty was encountered in obtaining anaërobes from these animals. Two types were obtained from the corn-and-protein and three from the corn-alone pigs. These organisms probably were not influenced by the diet.

The use of various forms of enrichment, such as a glucose fermentation tube, media containing egg white, $CaCO_3$, milk with and without fat on the surface, and several others, showed no difference between groups of animals.

A study of the percent of gas, gas ratio, percent of acid and sediment in fermentation tubes as suggested by Herter failed to solve the problem. Furthermore, tubes containing bile lactose, neutral red, etc., gave similarly disappointing results. Considerable work was done upon aerobic and anaerobic spore-forming types, but animals showed more individual variation within the group than was noted between groups. A study of the aciduric types failed to solve the problem. In most cases there were individual differences as great or even greater than those between groups. In general, it may be said that pigs on a corn diet show a slightly more simplified flora than pigs fed on a more complex diet.

STUDY OF THE B. COLI GROUP.

The *B. coli* group was next studied because it is an obligate intestinal type, and because of the work of Browne (12) upon this group. The group is admirably adapted to this work. Its characteristics are apparently quite stable, it is easy to isolate and cultivate, is one of the types found in the intestines of all the animals examined, and has been repeatedly reported by other workers on intestinal flora of hogs.

STABILITY OF CHARACTERS IN BACTERIA. It was necessary first to select the best method of handling the cultures and to determine features that would show actual stable variation rather than those due to laboratory manipulation. It is well known that certain organisms vary considerably upon culture media. As pointed out by Rogers, Clark and Davis (63), the value of any reaction to distinguish characteristics of organisms depends (1) on its usefulness in showing lines of natural relationship, and (2) on its stability.

The following is an extract from an article on the colon group of bacteria by Rogers, Clark and Davis, dealing with these points:

"It must be said that a character showing natural relationship would be stable, since stability comes through repetition in many generations. There is, or has been, considerable difference of opinion in regard to the stability of the various manifestations of fermentative ability in bacteria. Not a few writers asserted that the physiological reactions in general, and the fermentation of sugars in particular, are too variable to be used for purposes of classification. Burri found that old colonies frequently developed cells capable of producing gas from sugars not fermented by younger colonies. Gas formation was determined by shake agar cultures. Revis believes that physiological properties may be lost or acquired under action of competition and that a variation may become suddenly fixed. He found that when a typical *B. coli* was grown in a broth containing malachite green it gradually lost power of forming gas, although it grew luxuriantly and typically on solid media. This new variety seemed to be permanent. Penfold, in a series of papers, shows the possibility of bacterial mutations produced partly under the influence of chemicals added to the media and partly under normal conditions through the development of papillae on agar colonies. These new varieties which usually were nonfermenters were said to be permanent. On the other hand, Abbott, who was able to produce variation in *S. pyogenes aureus* by exposing repeated generations to various chemicals, found that this variation was in intensity of reactions rather than in the gain or loss of a function, and that the

sugar-splitting ability was not changed. Bergey, using similar methods with *B. coli,* was unable to obtain any mutations, although there was some evidence of alteration in some of the immunity reactions. Berry and Banzhof attempted to obtain by selection races of diphtheria bacilli with divergent powers of acid production, and found that the strains, instead of diverging, tended to approach each other. Similar results were obtained by Buchanan and Traux working with streptococci. Revis, who produced atypical varieties by exposing cultures to malachite green, found that in cultures of *B. coli* held several months in sterile soil and in synthetic media there was no loss of any physiological function, although there was some variation in the intensity of the reaction. MacConkey held *B. coli* in water 358 days without change in its characters. MacConkey also gives results of physiological tests on fifteen cultures of *B. typhosus* from different sources, including one that had been sixteen years on artificial media. All of these cultures gave identical reactions. Similar results were obtained by Harding, working with *Ps. campestris."*

Rougentzoff (65) compared indol production of fifty cultures of *B. coli* from normal rabbits and fifty cultures from starved rabbits and found them to be identical.

Remmelts (60) studied *B. coli* from cattle, hogs, sheep, goats, dogs, rats, mice, rabbits, guinea pigs, chickens and doves, and found only individual variations that did not show that they were distinct organisms.

It is also well known that *B. coli* may become quite strongly pathogenic under certain conditions. Its pathogenicity upon injection is, however, no indication of its pathogenicity upon ingestion.

Emmerich (24) and Korkunoff (44) could not produce infection from the intestine by feeding cultures of *B. coli.* Kartulis (40) could not produce infection by injection into the rectum, Jensen (38) succeeded in killing calves within a few hours by injecting, per os and per rectum, cultures of *B. coli* which he had isolated from calves suffering from severe diarrhea. Coppola (16) killed 20 per cent of his guinea pigs by introducing B. *coli* into the digestive tract.

As has been recently pointed out by various authorities, the problem of pathogenicity of *B. coli* is confused by the fact that *B. coli* does not represent a uniform type, but numerous varieties of a species, which show considerable differences in their morphological, cultural and biological characteristics. Also many organisms that have no relation to *B. coli communis* have been classified as such because they were isolated from the intestine and because of certain properties in common with *B. coli communis.*

As Kendall has said the intestinal organisms are "opportunists," potentially capable of becoming invasive whenever the barriers which ordinarily suffice to limit their development to the lumen of the alimentary canal become impaired, giving rise to endogenous infections.

The following summary of Browne's work upon the production of acid by the *B. coli* group may be made. The results obtained in this series of experiments seem to show that the source from which the members of the *B. coli* group were isolated had a direct effect upon the ability of the organism to ferment carbohydrates with the production of acid. The *B. coli* isolated from feces produced more acid in dextrose and lactose broth than the colon bacilli isolated from oysters, This seems to indicate that *B. coli* loses some of its ability to ferment carbohydrates with the production of acid during the journey from the intestinal tract to the oysters. Also, it indicates that *B. coli* isolated from the stools of laboratory assistants produce more acid in dextrose and lactose broths than similar organisms isolated from the stools of Italian emigrants. He states that the significance of these differences in the ability of the organisms, isolated from the above-named sources, to produce acid from carbohydrate is impossible to explain, except that the general character of the diet may have some effect on the ability of the organism to ferment carbohydrates with the production of acid. He further states that various members of the *B. coli* group, after remaining for eight weeks in bottles of sea water kept at 4° C. and 20° C., were able to produce the same amount of acid in the various carbohydrates as when first added.

Browne did not attempt to determine the effect upon the different varieties of the group, but has very good evidence that treatment does exert some influence upon the prominent biochemical characteristics of the organisms. Furthermore, this characteristic, when once established, is very constant. In the present work it was attempted not only to note the effect of diet upon the power to ferment carbohydrates, but also to note the influence of the diet upon the establishing of certain varieties of *B. coli* at the expense of others.

Levine (48), in his discussion of Browne's results, states that the Voges-Proskauer positive organisms, which he showed to produce less acid from dextrose, might have given

him the low results from his cultures from oysters. These are more common in sewage and soil washings, but rare in feces.

Greenfield has recently substantiated these results by using colon-like organisms from water, and ice.

Andrews (1) calls attention to the fact that the absence of sexual reproduction is one reason for the great variability among bacteria, and that binary fission may be a reason for not applying Weismann's principle of noninheritance of acquired characters to bacteria.

B. coli was selected for this investigation as the most prominent inhabitant of the intestinal canal. It is really an inmate of the' lower intestine, where it would be less influenced by variations in food and the inhibiting action of certain transient organisms. The writers thought that perhaps the long-continued diet might bring to light or abolish certain strains. Rettger noted, in the rat fed upon corn protein (zein), marked changes in the flora of the intestine, and attributed this to the indigestibility of the corn protein.

From the preceding it is evident that there are certain variations in bacteria due to their environment. Whether these characters are hereditary seems to be largely in doubt. There are, however, different species of bacteria in nature whose characters are stable within narrow limits under uniform conditions.

In the following described work the attempt was made to note differences between the colon organisms from the two groups of pigs by using the six carbohydrates described by Jackson and outlined in the Standard Methods of Water Analysis A. P. H. A. (1913). The results obtained were used to establish biometric relationships between cultures and to see how the different varieties of the colon bacillus are affected by the diet.

DESCRIPTION OF METHODS FOR THE STUDY OF THE B. COLI GROUP. In making the media for this work the standard methods for the examination of water and sewage were followed.

For the isolation of the colon types the method advocated by Kendall was used. This method is as follows:

A small portion of feces (one gram) is thoroughly emulsified in ten cubic centimeters of sugar-free broth, and preferably incubated one hour at 37° C. prior to the inoculation

of the plates. This preliminary incubation does two things; the clumps of bacteria settle down, leaving a more uniform suspension of bacteria in the supernatant fluid for inoculation, and the bacteria undergo a slight development in a medium particularly suited for their growth. The thin suspension of the stool is now rubbed upon the surface of the Endo agar plates by means of a bent, sterile glass rod, and the plates incubated for eighteen hours at 37° C. Colonies showing typical red reactions were isolated on agar slants.

To avoid as much as possible the variations which might arise on long cultivation of these organisms upon agar it was thought best to determine their powers of fermentation on carbohydrates as quickly as possible.

Owing to lack of proper equipment for the determination of the percent of gas and the gas ratio as recommended by Rogers and others, the writers were compelled to use the Smith fermentation tube. The percent of gas, the gas ratio and the percent of acid were determined in the usual way except that in the acid determinations it was attempted to obtain a more accurate idea of the total acidity by titrating cold. Recently boiled and cooled water was used for dilution; check determinations were always made, and these were subtracted from the data given. Standard meat infusion plus 1 percent Witte's peptone was used. In all cases 1 percent of the carbohydrate was added before sterilization. The media was sterilized fifteen minutes each day for three days and incubated twenty-four hours to avoid tubes showing contamination. The broth was neutralized to phenolphalein before tubing but usually was about .3 percent acid after sterilization.

While the writers are fully aware of the criticism of the method employed, and in general agree with it, they still feel that it will give valuable information in the absence of elaborate devices necessary for the determination of more exact data. To determine the relative experimental error in the use of the fermentation tube a series of sixteen tubes was inoculated and treated exactly alike. In the percent of gas the greatest difference between any two duplicates was 12 percent, with a mean probable error of ±4.4 percent. In the gas ratio the greatest difference was .202 percent and the percent of probable error for the whole series was ±10.5. In the acid determination the greatest difference between any two cultures

was .2 percent of normal acid and the percent of mean probable error was ±.21 percent.

As the attempt was to obtain comparative data upon groups of organisms rather than exact data upon any particular individuals, the experimental error in the determination does not obviate the results obtained by these methods.

The same person titrated all the cultures. Phenolphthalein was used as an indicator and a very faint permanent pink color was taken as an end point. The samples used were obtained from the fermentation tube. As it was possible to obtain only small amounts of dulcite and raffinose, small fermentation tubes containing about 3 cc. of media were used. In these one-half cc., obtained by using a long capillary pipette, was titrated with $\frac{N}{100}$ alkali. The same pipette was used in all cases and rinsed thoroughly each time with neutral distilled water.

All the cultures used were typical, within narrow limits, of the colon group. All organisms fermented glucose and lactose; all produced a red color on Endo media; all reduced nitrates; all except four produced indol; and all were short rods with round ends. No spores were seen. Some organisms were motile, other nonmotile, as determined by the hanging drop. Most were Gram negative. In all these cultures the Gram-negative organisms predominated, but in a few cultures some individuals retained the stain. This was thought to be a contamination, but careful replating of these cultures did not change this characteristic. Rogers, Clark and Davis report similar findings.

Except for a few cultures that failed to produce indol and a few cases in which very little acid occurred in milk, the cultures were typical of the colon group of bacteria.

In tabulating results the biometrical method of tabulation was used, in the hope of noting differences in the groupings as affected by different methods of feeding. The fermentation of various carbohydrates was selected as typical of the group. The fact that the group may be subdivided by this method led to its selection as a possible means of showing the influence of the diet upon the intestinal flora,

In the preliminary work the cultures from each animal were studied and grouped separately, but there was so much

variation between duplicate animals that it was necessary to average them.

In tables X, XI and XII is shown the distribution of cultures according to the percentage of total cultures showing a different amount of gas, a certain gas ratio, and different amounts of acid.

TABLE X.—*Distribution of Cultures Producing Different Amounts of Gas.*

PER CENT GAS.	0–5.	5–10.	10–15.	15–20.	20–25.	25–30.	30–35.	35–40.	40–45.	45–50.	50–55.	55–60.	60–65.	65–70.	70–75.	75–80.	80–85.	85–90.
PERCENT OF GAS FROM DEXTROSE.																		
C1110					5.3	10.5	15.8	47.3	15.8	5.3								
C					3.3	6.5	8.9	24.1	24.2	16.1	8.3	6.6	2.2					
CP				2.6	9.8	12.7	23.8	20.1	15.6	8.1	2.6	3.9						
PERCENT OF GAS FROM LACTOSE.																		
C1110				10.5	10.5	15.8	10.5	36.8	10.5	5.3								
C		1.4		3.3	22.8	20.7	18.1	15.4	11.2	4.8	1.0	1.7						
CP		4.3	1.3	2.9	12.1	19.0	40.9	16.7	2.6									
PERCENT OF GAS FROM DULCITE.																		
C1110			5.3					10.5	21.0		36.8	15.2	5.3			5.3		
C		10.0	3.0	6.0	2.2	1.9	4.5	6.9	17.4	13.6	19.9	7.5	6.4		0.5			
CP		3.5	1.4	6.3		7.6	5.8	14.0	26.1	15.4	13.0	4.1	1.4			1.4		
PERCENT OF GAS FROM SACCHAROSE.																		
C1110	89.4	10.6																
C	55.8	18.2	25.0															
CP	52.2	15.5	23.3		6.2				2.6					1.3				
PERCENT OF GAS FROM RAFFINOSE.																		
C1110	100																	
C	56.7		2.3		39.6			3.3										
CP	49.2	3.4	7.5		31.6			8.0										
PERCENT OF GAS FRCM MANNITE.																		
C1110								5.3	5.2	10.5	15.8	15.8	15.8	10.5	15.8		5.3	
C				1.2			3.8	1.7	4.5	8.3	9.3	23.2	24.5	12.2	8.3	2.7	1.0	
CP		3.1	3.1					2.8	10.1	9.3	11.0	16.1	20.5	14.2	5.9	3.1	1.4	

TABLE XI.—*Distribution of Cultures Showing Different Gas Ratios.*

Gas Ratio.*	.05–.10.	.10–.20.	.20–.30.	.30–.40.	.40–.50.	.50–.60.	.60–.70.	.70–.80.	.80–.90.	.90–1.00.	1.00–1.10.	1.10–1.20.	1.20–1.30.	1.30–1.40.	1.40–1.50.
FROM DEXTROSE.															
C1110.....	5.3	.....	16.8	16.8	32.6	21.0	5.3	5.3	.....	.....	.....	.....	.....	.....	.....
C.....	6.5	7.7	10.3	13.4	19.8	15.2	15.9	4.7	2.7	.....	1.4	2.4	.....	.....	.....
CP	2.6	5.4	16.9	18.7	17.5	11.8	14.8	.....	.....	11.9	.....	1.3	.....	.....	.....
FROM LACTOSE.															
C1110.....	5.3	15.8	47.3	21.0	10.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
C.....	4.2	28.6	30.2	25.7	10.3	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
CP	7.4	29.2	44.3	11.6	7.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
FROM DULCITE.															
C1110.....	5.2	10.5	63.1	15.8	5.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
C.....	8.9	18.1	32.5	31.5	7.3	0.5	1.2	.....	.....	.....	.....	.....	.....	.....	.....
CP	7.7	18.4	34.7	31.4	7.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
FROM MANNITE.															
C1110.....	.....	.....	31.6	47.3	10.5	.....	.....	.....	.....	10.5	.....	.....	.....	.....	.....
C.....	1.0	10.7	28.0	41.1	13.8	5.6	0.5	.....	.....	.....	.....	.....	.....	.....	.....
CP	6.7	10.1	28.2	42.2	9.5	3.3	.....	.....	.....	.....	.....	.....	.....	.....	.....

* Ratios obtained by dividing the amount of gas absorbed by alkali (CO_2) by the amount of unabsorbed gas (H_2)

TABLE XII.—*Distribution of Cultures Producing Different Amounts of Acid.*

PERCENT OF ACID FROM DEXTROSE.

PERCENT OF ACID.	0.0-0.19	0.2-0.39	0.4-0.59	0.6-0.79	0.8-0.99	1.0-1.19	1.2-1.39	1.4-1.59	1.6-1.79	1.8-1.99	2.0-2.19	2.2-2.39	2.4-2.59	2.6-2.79	2.8-2.99	3.0-3.19
C1110											6.3	31.3	16.8	16.8	11.4	16.8
C					1.2	1.0	2.8	0.5	6.5	13.4	18.9	24.3	14.9	9.6	4.4	1.4
CP			2.3		2.3		8.1	14.9	7.3	13.1	21.5	9.0	10.2	3.5	3.5	

PERCENT OF ACID FROM LACTOSE.

C1110						21.0	36.8	26.3	15.8							
C			0.5		1.4	11.6	19.7	33.6	17.6	1.7	4.2	1.0	1.0		1.3	3.7
CP						7.9	13.8	33.9	21.3	7.5		2.5				2.9

PERCENT OF ACID FROM DULCITE.

C1110				15.8	57.9	10.5	10.5	5.3								
C	4.9	1.8	4.6	19.9	18.5	15.0	13.5	12.4	4.7	1.5		4.9				
CP	2.9	2.9	7.7	13.5	37.8	23.1	6.3		5.9							

PERCENT OF ACID FROM SACCHAROSE.

C1110	78.4	15.8				5.3										
C	19.3	18.3	9.4	20.2	15.9	5.6	8.0	1.4		2.2						
CP	49.9	14.6	22.1	5.4	1.3	4.1			2.9							

PERCENT OF ACID FROM RAFFINOSE.

C1110	26.3	31.6	21.0	5.2	5.3	11.5										
C	22.8	11.4	10.4	10.4	7.9	15.2	11.3	5.4	1.3	6.5						
CP	30.0	10.7	1.3	9.8	25.1	10.7	10.4		1.3							

PERCENT OF ACID FROM MANNITE.

C1110				31.6	42.1	15.8	10.5									
C		1.0	1.0	28.8	33.1	21.4	8.8	4.9	0.5	1.0		0.5				
CP				18.7	36.3	24.1	17.2	4.1								

In arranging these tables the averages from all the pigs fed on corn alone were obtained, except for pig C1110, and from the pigs fed on corn plus milk albumen. A comparison of these groups was madewith C1110, as this animal had been on a corn diet for a much longer period. If the diet has a marked influence upon the flora it should show in this animal.

PERCENT OF GAS. It is very difficult to discuss the results obtained. Dextrose does not differentiate the groups of animals, except that in the case of pig C1110 the organisms studied are grouped more closely, nearly all producing 40 percent of gas.

In lactose the cultures from pig C1110 tend to group more closely than the others and produce a higher percent of gas. In dulcite there are many scattered groups and in the corn-alone pigs and pig C1110 there are two large groups, one producing about 45 percent of gas and one producing about 55 percent of gas.

Raffinose shows practically no organisms producing gas from pig C1110. The corn and corn-and-protein pigs show a similar distribution. One point of interest is that in each of these groups of animals there is a group of organisms producing about 20 percent of gas. This group is not shown by pig C1110.

Saccharose shows very little except that there are a small number of organisms from the corn-and-protein pigs able to produce gas. These are not shown by pig C1110.

Mannite shows the organisms from all the pigs are much the same. In pig C1110 the organisms are more closely grouped and show a tendency to produce slightly more gas.

GAS RATIO. The gas ratio shows nothing as to the separation of the groups of animals. There is a slight tendency for the organisms from pig C1110 to be grouped more closely than in the case of the corn-alone and corn-and-protein groups. According to Rogers and others, 'these are not the correct ratios as given by colon bacilli. Theyare much too low, showing that considerable CO_2 was lost.

PERCENT OF ACID. These figures show the same tendency to group the organisms from pig C1110 somewhat more closely than in the case of the other animals. Also, there are fewer

TABLE XIII.—*Relation Between Acid and Gas Production.*

Carbohydrate.	Gas.	Group.	Percent cultures showing gas.	Percent of acid.									
				.0–.19.	.20–.39.	.40–.59.	.60–.79.	.80–.99.	1.00–1.19.	1.20–1.39.	1.40–1.59.	1.60–1.79.	1.80–2.00.
SACCHAROSE.	+	C1110....	10.5	0	50.0	0	0	0	50.0	0	0	0	0
	+	C.....	32.7	5.2	5.4	8.1	24.3	27.0	8.1	13.5	5.4	0	2.7
	+	CP.....	50.0	3.7	29.6	48.1	3.7	0	7.4	3.7	0	3.7	0
	−	C1110....	89.5	66.6	33.4	0	0	0	0	0	0	0	0
	−	C.....	67.3	40.3	18.1	10.5	13.3	9.1	6.1	1.3	0	0	0
	−	CP.....	50.0	28.8	0	3.7	3.7	3.7	0	0	0	0	0
RAFFINOSE.	+	C1110....	0	0	0	0	0	0	0	0	0	0	0
	+	C.....	37.1	0	2.9	2.9	5.8	16.4	43.5	17.4	11.6	0	0
	+	CP.....	55.0	0	3.0	0	12.1	36.4	21.2	24.2	0	3.0	0
	−	C1110....	100.0	24.8	31.1	24.9	6.2	6.2	6.2	0	0	0	0
	−	C.....	62.9	25.8	22.1	19.7	20.9	2.4	2.4	3.6	0	1.2	1.2
	−	CP.....	45.0	48.1	25.9	3.7	3.7	11.1	0	7.4	0	0	0

organisms from this animal able to attack saccharose and raffinose with the production of acids.

In several cultures, however, quite appreciable amounts of acid were produced in saccharose and raffinose when no gas was formed. Some of these cultures were tried several times with the same result. Table XIII shows the relation between acid and gas production in saccharose and raffinose.

The data in this table were obtained by using 19 cultures from pig C1110; 116 cultures from the corn-alone group; 67 cultures from the corn-and-protein group. From the data a marked tendency may be noted for the organisms producing acid to produce gas also. This is especially true in the case of the corn-fed animals. In considering the cultures from pig C1110 10.5 percent were able to produce gas from saccharose and none able to ferment raffinose with production of gas. In the corn-alone group 32.7 percent were able to produce gas in saccharose and 37.1 prcent were able to produce gas in raffinose, On the other hand, 50 percent of the cultures from the corn-and-protein group were able to produce gas in saccharose and 55 percent in raffinose. There is some tendency for the corn-alone and the corn-and-protein pigs to produce higher amounts of acid in raffinose than in saccharose. Tables XIV, XV and XVI show averages of all cultures from the different groups of animals.

TABLE XIV.—*Average Per Cent of Gas.*

	Carbohydrates.					
	Dextrose.	Lactose.	Dulcite.	Saccharose.	Raffinose.	Mannite.
Pig C1110	41	38	58	Trace.	0	64
Corn-alone pigs	46	36	54	Trace.	Trace.	55
Corn-and-protein pigs	44	35	47	1.5	18	59

TABLE XV.—*Average Gas Ratio.*

	Carbohydrates.					
	Dextrose.	Lactose.	Dulcite.	Saccharose.	Raffinose.	Mannite.
Pig C1110	.51	.44	.39	—	—	.38
Corn-alone pigs	.56	.38	.36	—	—	.43
Corn-and-protein pigs	.55	.31	.35	—	—	.40

TABLE XVI.—*Average Per Cent Acid.*

	Carbonydrates.					
	Dextrose.	Lactose.	Dulcite.	Saccharose.	Raffinose.	Mannite.
Pig C1110	2.83	1.47	1.06	0.10	0.51	1.03
Corn-alone pigs	2.29	1.68	1.14	0.69	1.02	1.08
Corn-and-protein pigs	2.35	1.91	1.05	0.60	0.85	1.06

PLATE II.

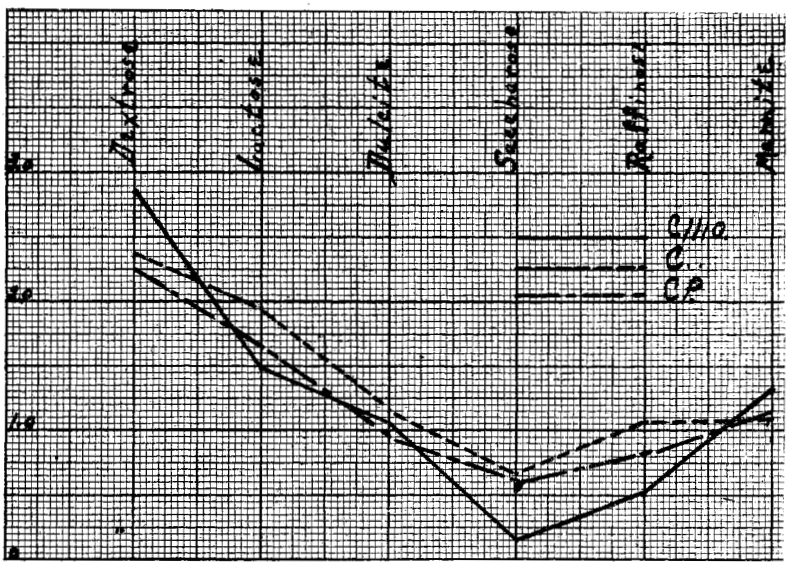


Plate II shows the relation of colon cultures from different groups of pigs in their ability to ferment the different carbohydrates with the production of acid. Cultures from pig C1110 fermented dextrose and mannite with the production of more acid, while they fermented saccharose and raffinose with the production of less acid than the other groups. In all carbohydrates the corn-alone pigs produced slightly more acid than the corn-and-protein pigs.

VARIETIES OF B. COLI STUDIED. In summarizing the data obtained, the writers were unable to find anything that would distinguish the groups of animals except, perhaps on the basis of types. With this in mind the cultures were arranged according to Jackson's classification (35). He classified the

colon group of organisms according to their ability to ferment certain carbohydrates as follows:

1. *B. communior* (Durham) with five varieties.
2. *B. communis* (Escherich) with five varieties.
3. *B. ærogenes* (Escherich) with five varieties.
4. *B. acidi lactis* (Hueppe) with five varieties.

By using the above classification all the cultures were arranged under the various groups. The figures given are in percent of the entire number of cultures. These were calculated on a percentage basis, so that they might be more easily compared, as no two groups of animals had the same number of cultures.

TABLE XVII.—*Showing the Distribution of Different Varieties of B. coli as Influenced by Diet.*

No Cultures.	Pig.	*B. communior* A1.	*B. communior* A2.	*B. communior* B.	*B. communis* A.	*B. communis* B.	*B. ærogenes* A2.	*B. acidi lactici* B.
19	C1110	0.0	0.0	5.2	0.0	94.7	0.0	0.0
67	C1	4.3	2.9	7.1	0.0	84.2	0.0	1.4
25	C100	66.7	1.5	4.3	3.5	25.0	0.0	0.0
24	C1000	39.1	0.0	7.1	2.3	47.7	4.3	0.0
40	CP1111	52.4	0.0	7.1	4.9	33.3	0.0	2.4
17	CP1210	41.2	0.0	0.0	0.0	58.8	0.0	0.0

TABLE XVIII.—*Showing the Distribution of Different Varieties of B. coli in the Corn and Corn-and-Protein Pigs.*

Pig.	*B. communior* A1.	*B. communior* A2.	*B. communior* B1.	*B. communis* A.	*B. communis* B.	*B. ærogenes* A2.	*B. acidi lactici* B.
C1110	0.0	0.0	5.2	0.0	94.7	0.0	0.0
C1, C100, C1000	36.7	3.2	3.8	2.9	52.3	1.4	0.4
CP111, CP1210	46.3	0.0	3.5	2.4	46.1	0.0	0.8

Very little work has been done to show the influence of a diet upon the elimination of varieties of the *B. coli* group. Kendall (41) mentioned that from the jejunum to the ileocecal valve numbers of *B. lactis ærogenes* group occur most commonly. This was not true of the two slaughtered pigs examined. Of the fifteen cultures from this part of the intestines of pig C1, none were *B. lactis ærogenes* and on the one

trial on pig CP1111 this organism was not isolated in twelve cultures. This, however, is too small a number of animals to prove this point definitely even in case of pigs.

In the case of the corn-alone pigs there is a tendency for the *B. communis* group to predominate, while in the corn-and-protein pigs the *B. communis* and *B. communior* groups are about equal. This is more marked in some animals than in others, especially in the case of pig C1110. It may be that the long-continued corn diet has crowded out some of the more highly saprophytic type of *B. cummunior* for a more highly parasitic *B. cummunis* type. It may be that this is an idividual variation, as not enough animals were used to prove this point.

It will be noted in table XVII that there is a great variation between the individual pigs in each group, Pig C1110 is not included in the average, as this animal belonged to another lot. In pig C1 the *B. communis* variety greatly predominates. This is also true, but to a less extent, in pig C1000. In pig C100, fed in exactly the same manner, the *B. communior* group predominates. In pig C1110 the *B. communis* group greatly predominates.

In pig CP1111 the *B. communior* group predominates to some extent, while in pig CP1210, fed in exactly the same manner, the *B. communis* group predominates.

In the averages, the corn-alone pigs showed a slightly greater number of *B. communis,* while in the averages for the corn-and-protein fed pigs the groups are equally distributed. There seems to be a marked tendency, when corn alone is fed for a long time, to increase the *B. communis* varieties at the expense of all others. This point is well illustrated in case of pig C1110.

The various other types are more or less equally distributed, but are present in such small numbers as to be of little significance. These were not found at all in pig C1110.

Due to the great dominance of a *B. communis* in pig C1110 after about twenty months on corn alone, it was decided to investigate the influence of a change in diet. Thus twenty colon cultures were isolated from this animal and twenty from pig C111, which had been fed, since weaning, on corn plus ash.

Of these twenty cultures from pig C1110, nineteen proved to be *B. communis,* while all the cultures from pig C111 were *B. communior.*

The ration of these two animals was then changed to full feed of corn, shorts and tankage. After thirty days twenty cultures from these animals showed that ten of the cultures from pig C1110 were *B. communis* and ten were *B. communior.* Of the cultures from pig C111 four were *B. communior* and sixteen were *B. communis.*

The above shows that in these cases, at least, the diet did have a marked influence upon certain types of colon bacteria in the intestine. The nutrition part of the experiment was closed before further work could be done on this phase of the problem.

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