NOTES UPON AN EPIDEMIC OF FOWL CHOLERA AND UPON THE COMPARATIVE PRODUCTION OF ACID BY ALLIED BACTERIA.

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The bacillus of fowl cholera, although previously observed by Perroncito and others, was first successfully cultivated in 1880 by Pasteur, whose investigations of this organism mark an epoch in the bacteriological study of disease. Although this bacillus is well known and has been fully described, it has not been positively identified as the causal factor of disease upon this continent. Cholera in poultry, it is true, exists here, and in Bulletin No. 8 of the Bureau of Animal Industry there is to be found a very careful study of "Three Outbreaks of Fowl Cholera" by Dr. V. A. Moore. These epidemics occurred in places far apart, one near Providence, R. I., another in Virginia, and another near Washington. The germ from all three proved to be the same. It differed, however, in its characters from the European bacillus of fowl cholera, though closely allied thereto. The germ that I am about to describe would seem closely to resemble, if not to be identical with, the true bacillus of fowl cholera as found in various European countries.

The fact, then, that I have been able to isolate a germ which, while closely related to that causing outbreaks of a very similar nature along the Atlantic coast, has been found after prolonged study to be distinct from this form, would seem to be sufficient ground for publishing a description of the organism and its characters. In studying this form I have been enabled to pursue a series of investigations upon certain methods for the differentiation of species. Although I cannot state that these studies have led to complete success, it will, I think, be well to mention those employed by me, so that others may

know how much has been done along these lines and with how much success, and may possibly carry on the work to a more successful conclusion.

I have more especially attempted to arrive at methods for the study of the comparative acid production by allied forms of bacteria, employing germs of various virulence from this particular outbreak, together with forms isolated from the epidemics of fowl cholera in the States and cultures of the bacillus of the disease received from Europe. To gain a sure basis for such investigations I have endeavored to manufacture synthesized media so that I might more surely start from known compounds capable of being split up by bacterial growth. The number of synthesized media that I have produced* has been very great, but my success in the matter has only been partial, for the bacillus of fowl cholera and its allies grow but poorly upon nearly all the fluids made up by me. Nevertheless, I feel that even my partial success has given me fuller knowledge of this subject of acid production by bacteria, and the results obtained may possibly be deemed of value as throwing some light upon at least one more possible method of differentiating between closely allied forms.

My attention was called to this outbreak December 5, 1895, by Professor Adami, who had in turn heard of it through Dr. G. P. Girdwood. The outbreak occurred among a flock of fowls belonging to Mr. A., of St. Anne's, P. Q. St. Anne's is situated about 20 miles from the city of Montreal, on the island of Montreal, where the St. Lawrence divides to encircle the island. On the 8th I visited Mr. A., among whose fowls the outbreak had occurred.

History.—The first signs of the disease had been noticed about a month previously, the animals dying very suddenly. The disastrous results were communicated to Mrs. G., among whose fowls an outbreak had occurred several years previously. She suggested the placing of sulphuric acid and sulphate of iron in the drinking water and allowing access to no other water, also a complete disinfection of the hen-house. After cleansing the coop and administering the above remedy the disastrous results were rapidly checked, but few fowls having died since. The disease pursued a very rapid course, producing death in from 3 to 12 hours from the time that the first signs were noticed. The greater

* See addendum to this paper.

number were found dead in the morning (from two to four at a time). In all there were lost about 20 fowls, which were invariably among the youngest. The older ones seemed immune and none showed symptoms of the disease.

Symptoms.—From the outset there was a profuse diarrhœa, yellow in color; the feathers were ruffled and there was a yellowness about the head. The fowls were not inclined to move after noticeable symptoms occurred, but rather showed a tendency to roll themselves up in a ball, so to speak, and to remain motionless till they fell over dead.

The hygienic conditions were favorable for the development of any infectious disease. The fowls were confined in a coop about ten feet square and six feet high. There were two small windows, one on the east and the other on the west elevation, each being about two feet square. At the time of my visit the place was damp and was said to have been particularly so at the time of the outbreak. After the outbreak a fire was started in order to dry out the apartment. There was little or no ventilation.

Three animals dead of the disease in question were procured. The exact length of time they had been dead was not definitely known, but it was thought to have been at least a week. Upon the death of the fowls they were immediately thrown outside and were at the time of the visit covered with snow. For several days it had been very cold; there was a snow-storm on December 2nd and another the night before the visit (that of December 7th). To all appearances the fowls had been covered by both falls of snow. Probably they had been exposed during the time they had lain outside to a mean temperature of at least — 20° C., it having been very cold after the first fall of snow. On the night of the 8th they were left in a sleigh out of doors, a thermometer nearby registering —16° F. or —27° C. at 8 A. M. on December 9th. They were brought to the Molson Pathological Laboratory of McGill University on the morning of the 9th.

Morbid Anatomy.—The appearances found at the necropsy will be cited for but one case, as there was little or no difference noted in the three. The subject was a mongrel Plymouth Rock hen about eight months old. She was in good condition. The liver was rather pale with areas of necrosis. Spleen soft, pulpy and enlarged. The intestines were pale externally and free from evidence of hæmorrhage. Within, the mucosa was congested and swollen. The lower portion of the intestines was particularly affected, and its follicles were moderately distinct. There was fluid in the pericardial cavity, with some hæmorrhage beneath the

pericardium, and marked bloody imbibition of the vessel walls of various organs.

Cultures were made from various organs and fluids of the body, and revealed on the following day a pure and moderately vigorous growth of a non-motile bacillus. The bacillus in all the cultures from the same fowl appeared to be identical; the cultures from the various fowls were found to be identical and pure.

DESCRIPTION OF THE BACILLUS.

Morphology.—The micro-organism is a non-motile, rod-shaped organism, aerobic, though growing in the presence of little free oxygen, varying slightly in size according to the medium upon which it is grown. In broth at 37° C., at the end of 20 hours, the bacilli vary from 1.3 to 1.8 μ in length and from 0.5 to 0.7 μ in breadth. The most frequent form is a diplobacillus with rounded ends, which may under low magnification be mistaken for a diplococcus. In the hanging-drop preparation it shows a distinct polar arrangement of the protoplasm. It is frequently seen in old cultures in short chains consisting of three or four of the bacilli placed end to end. It stains with the ordinary aniline dyes but retains the coloring matter feebly or not at all when subjected to Gram's method. It appears in great numbers in the tissues and also in the blood. In the tissues it often occurs in clumps of four or five. No spores or any tendency to spore-formation have been recognized. A feebly-staining capsule has been occasionally observed.

Cultures in Broth.—This medium was made by using 2 grammes of Liebig's extract to 500 cc. of distilled water, 1 per cent Witte's peptone and 0.5 per cent rock salt. Kept at 37° C. the broth is uniformly clouded at the end of 24 hours. This cloudiness does not as a rule settle, but in some cases the broth eventually cleared, the conditions being kept unaltered. The germ was found to be still alive in tubes which had stayed in the laboratory for 9 weeks.

When ordinary broth is neutralized with hydrochloric acid or sodium hydrate, as the case may be, phenolphthalein being used as an indicator, the germ will grow between the limits of 4 per cent of a decinormal solution of hydrochloric acid and 4 per cent of a decinormal solution of sodium hydrate. Between these two limits the culture shows very little difference upon removal from the incubator at the end of 24 hours. Beyond these two points, on both the acid and alkaline sides, the growth is very feeble, and on tubes containing 8 per cent of decinormal hydrochloric acid and of those containing 10 per cent of decinormal sodium

hydrate solution there is no growth at the end of 24 hours. It may be added that there is a decided odor characteristic of broth cultures of this germ.

Fermentation (gas production) experiments by means of the Smith tubes proved negative with both 1 per cent lactose and 1 per cent glucose broth.

Nutrient Gelatine Cultures.—Here the Liebig's extract broth was used, 7 per cent of gelatine being added. This when made up was slightly acid (to phenolphthalein), but the acidity was well within the limits determined by the previous experiments with broth cultures.

The growth on the nutrient gelatine is less vigorous than in broth and is recognizable in stab cultures and on plates about the third or fourth day. In stab cultures there is little or no growth at the surface, the growth being equal all along the line of inoculation. On plates, superficial colonies about 0.5 mm. in diameter are seen on the third or fourth day; the deep colonies are more minute. The surface growths are granular without sharply-defined borders. The deep colonies show a granular appearance; their color is a very pale white. There is no liquefaction. It was noticeable that no cultures were obtained beneath mica plates. In other words the bacillus will not grow when deprived of free oxygen, but grows better where there is little oxygen (along the stab in stab cultures) than where oxygen is abundant (on the surface).

Agar Cultures.—The Liebig's extract broth was used for this purpose, 1 per cent of agar being added. This also was slightly acid to phenolphthalein, but the acidity was well within the limits of active growth. Growth on this medium at 37° C. is not very vigorous; at the end of 24 hours there appears a very pale white, almost colorless streak along the line of inoculation which has a glistening appearance. Isolated colonies are about 0.2 to 0.5 mm. in diameter, convex with granular edges. There is acid production, but this is not abundant. Lactose litmus-agar shows a red coloration along the streak on the second day. Only after about 4 days is there a reddening of the surrounding medium, and even then this is not very extensive. A peculiar penetrating odor arises from both tubes and plates.

Potato Cultures.—Upon this medium the growth is not very vigorous, but is present as a slight yellowish coloration along the line of inoculation after 4 days.

Milk.—A change is noted in the consistency of the cream after 4 days. Other than this no change is to be recognized in milk cultures kept in the incubator for 6 weeks.

Blood Serum.—Natural blood serum coagulated was used. The growth is very similar to that obtained upon agar, but is not nearly so vigorous. The growth along the line of inoculation is seen after a few days to broaden slowly if the tube be kept in the incubator.

Temperature Relationships.—The germ grows at ordinary temperature and also at that of the blood. It resists a heat of 59° C. for 10 minutes; it will not withstand one of 60° C. for that length of time. These relationships were determined according to the instructions laid down in Sternberg's Text-book of Bacteriology, 1896. As already pointed out by me the germ is evidently capable of resisting a temperature below 0° C. for several days and of —27° C. for several hours, although evidently this extreme cold led to some attenuation.

Action of Disinfectants.—A 1 per cent solution of carbolic acid destroyed the life of the germ in 5 minutes. A half per cent solution of hydrochloric acid was fatal in the same time, as was also a half per cent solution of sulphuric acid. The commercial hydrochloric and sulphuric acids were employed in these experiments.

RESULTS OF INOCULATION.

Poultry.—A fowl inoculated intravenously with 2 cc. of a culture 24 hours old was found dead on the second day. Further than this the virulence for fowls has not been determined, on account of an accident, owing to which the virulent germ was lost. A few hours after inoculation the fowls became listless. Even with the most attenuated virus a few hours after inoculation a profuse diarrhœa was noticed, the stools consisting of semi-mucoid masses of a greenish-yellow or white color. The autopsy showed as follows: Intestine filled with gas, crop empty, gizzard filled with small stones and ingesta. Intestine contained a greenish-yellow, semi-mucoid mass, kidneys normal, spleen congested and enlarged. Kidney fatty. Gall-bladder much distended. Heart and lungs normal. Where the fowls had been given a subcutaneous injection of a less virulent form into the breast muscle, at the point of injection was a mass of cartilaginous hardness, of a yellow color, free in the muscle tissue. The bacilli were found in all the fluids and tissues of the body.

Immunity has been produced in young chickens by the injection of a moderate quantity of an attenuated germ.

Turkey.—A turkey was inoculated intravenously with 6 cc. of a 24-hours-old culture and showed symptoms about an hour afterwards. At this time he was loath to stand; the feathers were slightly ruffled and the head drawn close to the body. The symptoms further than this

were not noted, as the animal was inoculated at night and found dead in the same spot on the following morning, having succumbed in about 15 hours. The autopsy, made a few hours after death, revealed the following: Liver, great congestion, otherwise normal. Heart, lungs, spleen and kidneys normal. Intestine, pale externally. From within, the mucosa was seen to be swollen and greatly congested, showing ecchymoses and areas of necrosis. This congestion was not localized, but extended from the gizzard to the anus. The intestine was filled with partially digested food of a greenish color. Crop greatly distended with ingesta. The mucous surfaces, from the mouth to the gizzard, were studded with ecchymoses and showed congested areas. This was also true of the mucous surfaces of the cavities of the head. The mucosa of the gizzard also showed congested areas.

*Rabbits.**—The first rabbit was inoculated through the digestive tract, and death occurred on the twelfth day. The disease followed the course of a septicæmia, the symptoms of which were characteristic. Another animal inoculated with a culture, 24 hours old, from this first died two days later. After this the germ rapidly gained virulence, the last rabbit, which was the ninth of the series, dying 6 hours from the time of inoculation. This last rabbit was inoculated with 3 cc. of a preparation of ten drops of blood from the previous animal to 10 cc. of broth. The lesions presented at the autopsy were as follows: Upon opening the abdominal cavity there was noted acute peritonitis with a slight effusion of serum. The intestine was distended with gas. The mucosa of the stomach peeled off with ease, the surface being studded with small ecchymoses and congested. The intestine was filled with a greenish-yellow, semi-mucoid mass. The mucosa was injected. Appendix discolored, mucosa congested with small ecchymoses. The large intestine contained gas and a dark semi-fluid mass. Liver firm, gall-bladder greatly distended. Kidneys slightly congested. Heart and lungs normal. The mucosa of the uterus showed great congestion and partial necrosis. The bacillus was found in the blood, and cultures from the various organs also revealed its presence. In this case it was demonstrated in the vitreous humor of the eye.

The symptoms of the inoculation disease in rabbits were marked. There was drowsiness. The hind legs were drawn well under the body.

* Evidently the conditions under which the bacilli were gained, namely, from the bodies of animals which had been dead for several days, and had been preserved at a temperature far below 0° Centigrade, had induced a very definite attenuation of the microbes.

The forelegs were drawn as far back as possible. In the disease produced by the virulent germ there was profuse diarrhœa beginning a few hours after inoculation, and in those withstanding for some time small tubercle-like masses were found in the appendix. Rabbits inoculated with the most virulent virus developed symptoms of an acute disease, remaining motionless until death supervened. In the disease produced by the attenuated germ the symptoms were similar, but the animal took food up to about 24 hours before death, at which time acute symptoms developed.

A guinea-pig inoculated with 1 cc. inside the thigh developed a local abscess.

Dogs fed on dead subjects developed no marked symptoms save a profuse diarrhœa. There was no elevation of the temperature.

The bacillus here described corresponds closely in all respects with the bacillus of European chicken cholera. So close is the resemblance that I cannot but hold that the two are identical, and that I have been dealing with an epidemic of the true disease, the first recorded in America. In only two respects have I found slight divergences from the statements of previous observers, namely, in the resistance of the organism to heat and disinfectants. The European germ is said by Baumgarten to be destroyed by an exposure to 55° C. for fifteen minutes or, according to Salmon, by an exposure to 56° C. for ten minutes. The form here described was killed only by a temperature of 59° C. with ten minutes' exposure. On the other hand it would appear to be more sensitive to disinfectants, for whereas Hueppe found that the European germ was killed by 3 per cent carbolic acid in six hours, the Canadian form did not grow after being left in 1 per cent carbolic acid for five minutes. The discrepancy in both cases would appear considerable, but it must be remembered that the want of agreement in the results of various observers in connection with these two tests of thermal death-point and disinfectant action are notorious, so that, when these two tests alone yield divergences, we do not possess sufficient grounds for stating that we are dealing with separate species.

From the form already recognized in America the separation is clearly marked. The Canadian germ is smaller, takes the polar stain

well, does not saponify milk, produces acid in dextrose and lactose broths, can be rendered most virulent for rabbits so that a drop or two of the blood of an affected rabbit will lead to the death of another in six hours, while both in fowls and rabbits there is developed a most profound diarrhœa. In addition to these differences I have found, in comparing the growths, that the American form flourishes much more vigorously in gelatine (stab and streak cultures), so that the growth is already recognizable in twenty-four hours, while the Canadian and European forms show nothing until the fourth day. On the other hand in plate cultures the colonies of the American form are much smaller, with more sharply-defined borders. There is little or no difference upon agar.

The study of the whole of the large group of bacilli of hæmorrhagic septicæmias has revealed so much variability, so many individual points of difference between microbes in the main closely resembling each other, that I shall not venture to say that the forms isolated by Dr. Moore belong to a distinct species. I only urge that the Canadian form approaches much more nearly to the classical type.

Before closing this section of my paper attention may be called to what, I believe, has not previously been noted, namely, that the turkey is very susceptible to inoculation chicken cholera.

UPON THE COMPARATIVE PRODUCTION OF ACID BY ALLIED BACTERIA AS A MEANS OF SPECIES DIFFERENTIATION.

In studying these three allied forms of bacteria I was led, thanks to Professor Adami, to pass beyond the limits of my initial research. His suggestion was that the comparative acid and alkali production by various species of bacteria had been insufficiently studied and that possibly a careful examination of such acid and alkali production might reveal a further means of differentiating allied forms. Acting upon this idea I have spent several months endeavoring to arrive at satisfactory conclusions with regard to the subject. Even now I cannot consider that more than preliminary studies have been made, nor should I publish at the present time were it not probable that an indefinite period may elapse before I can again take up this subject, while it is possible

that the record of my results obtained thus far may be of immediate use to others about to enter into like investigations.

In the first place I found that the fowl-cholera organisms resemble most of the pathogenic microbes in producing acid during the earlier stages of their growth.

To determine the amount of acid quantitatively the media had to be carefully prepared. In the earlier experiments meat infusion was employed, made strictly in accordance with the directions given by Abbott in his *Principles of Bacteriology*. This broth was neutralized with sodium hydrate, phenolphthalein being employed as an indicator. That no appreciable amount of muscle sugar was present in the broth so prepared was determined by the fermentation-tube test, the bacillus coli being employed as the testing germ.* Four tubes were then taken containing 10 ccm. of the neutral broth and were sterilized in the autoclave. Of these, two were inoculated with the germ; the other two were used as controls.

The following is an example of the result of titration at the end of 24 hours' growth at 37° C. The figures given indicate the number of cubic centimetres of a decinormal solution of sodium hydrate required to neutralize the broth, phenolphthalein being here, as elsewhere, employed as the indicator:

TABLE I.

Tube inoculated with Canadian chicken cholera.	No. 1	1.00
" " " " " "	" 2	1.10
Sterile tube, not inoculated.	" 3	0.15
" " " "	" 4	0.20

It will be seen that the process of sterilization of the broth in the tubes after the broth had been neutralized, and the subsequent keeping of these tubes at 37° C., had led to a slight acid production in the broth, but the amount was very small as compared with the acid production in the inoculated tubes, in each of which the amount of acid was almost the same.

I had prepared a large quantity of the broth made from meat infusion, and used in the above series of experiments, and part of it I next employed for a fuller experiment. Taking 20 tubes I placed in each, as before, 10 cubic centimetres of the neutral broth and then sterilized.

* Theobald Smith's ready method of making sugar-free bouillon (*Journal of Experimental Medicine*, ii (1897), 546) was published subsequent to the completion of this paper.

Three of the tubes were inoculated with a diplobacillus, sent to me by Dr. V. A. Moore and stated to have been isolated from diphtheritic lesions in fowls. Three were inoculated with the United States bacillus of fowl cholera, for which again I am indebted to Dr. Moore. Four were inoculated with the Canadian fowl cholera, four with anthrax, while five were not inoculated and were carried through as controls. In order that the amount of culture placed in each tube of the series might be the same, and might contain the same number of bacilli, I employed, not the platinum loop, but Pasteur pipettes, drawn out into fine capillary tubes. These were sterilized, and for each series one was filled with a 24-hour-old broth culture of one or other germ, and from it one drop was allowed to fall into each tube to be inoculated. Tubes so inoculated were placed in the incubator for 24 hours, at the end of which time they were titrated in the usual manner. Here again, in the table that follows, the figures given indicate the number of cubic centimetres of a decinormal solution required to neutralize the broth. It will be seen that the control tubes were in this case slightly alkaline and required an addition of acid for neutralization. Evidently the neutralization had originally been carried a little towards the alkaline side.

TABLE II.

Tubes.	1.	Uninoculated, employed as control	0.1
	2.	" " "	0.1
	3.	" " "	0.0
	4.	" " "	0.1
	5.	" " "	0.1
	6.	United States fowl diphtheria	0.3
	7.	" " " "	0.1
	8.	" " " "	0.05
	9.	United States fowl cholera	0.75
	10.	" " " "	0.4
	11.	" " " "	0.3
	12.	Canadian chicken cholera	0.5
	13.	" " "	0.5
	14.	" " "	0.6
	15.	" " "	0.6
	16.	Anthrax	0.2
	17.	"	0.15
	18.	"	0.2
	19.	"	0.2

It cannot be stated that the results of these experiments were entirely satisfactory. In two forms, namely, in the two cultures obtained from

Dr. Moore, there was a variation in the acid production, so wide, indeed, that I have been unable to account for it satisfactorily. The cultures in all cases were pure, and the only marked difference that I could make out between the various growths in these two series was that the test tubes, while containing each 10 ccm., were of unequal diameters, so that in some cases a larger and in others a smaller surface was exposed to the atmosphere. Another possible cause for this irregularity may be the fact that whereas the chicken cholera and anthrax germs had been constantly transplanted, the other two had not, having been but recently transplanted from tubes of some little age sent from Washington.

Upon endeavoring to repeat these observations and to reproduce a broth identical with that employed in this last experiment I found that the amount of neutralization, the presence of muscle sugar in minute quantities in the different broths, and still other differences rendered it almost impossible for me to hope to gain any scientific precision in my results by following the lines employed up to this time. Hence it became necessary to endeavor to settle the question of acid production by different species and by allied forms of the same species by the employment of accurately compounded synthesized media.

In pursuing this work I have tried the formulæ of most of those who have already endeavored to form satisfactory synthesized culture media, and have further studied some fifty media made up in accordance with my own ideas. The work was very interesting, though at times very discouraging. It was my aim not so much to arrive at the formula of a medium specially suitable for the fowl cholera germ, but rather one which could be substituted for ordinary broth in ordinary work. There exist several media upon which other pathogenic germs, like those of anthrax and the bacillus pyocyaneus, will grow, but singularly few upon which the bacillus of chicken cholera will flourish. This difficulty in getting the bacillus of chicken cholera to grow in these media is wholly in harmony with the observations of Pasteur, who noted that it would not grow in various vegetable infusions. The great difficulty is in the addition of those elements or salts which are required in very minute quantities, as again in the proper addition of the calcium and magnesium salts. These latter are inclined to precipitate at an inopportune moment, a most undesirable feature. Not being able therefore to gain any satisfactory synthesized media, I was, for this work on chicken cholera, finally compelled to fall back again upon the ordinary broth. Liebig's extract of beef was now used in place of meat infusion, for I have found it less liable to contain fermentable matter. Four grammes of the ex-

tract were taken, dissolved in distilled water; 1 per cent of Witte's peptone and ½ per cent of rock salt were added. These ingredients were thoroughly dissolved, neutralized to phenolphthalein with a caustic potash solution and sterilized, the tubes filled and once more sterilized. In all cases the media were tested in the fermentation tube with the bacillus coli to determine the absence of fermentable matter. All the media used so far have given a negative reaction.

With this medium the first results obtained appeared to be hopeful and to give further proof of the close relationship between the European and Canadian forms. Thus I inoculated three tubes with equal quantities of a culture of the Canadian germ and three from a culture of the European form from the laboratory of Professor Kitt in Munich and obtained the following results:

TABLE III.

1.	Uninoculated tube as check	0.2
2.	" " "	0.2
3.	Canadian chicken cholera	0.4
4.	" " "	0.4
5.	" " "	0.3
6.	European fowl cholera from Munich	0.3
7.	" " " " "	0.3
8.	" " " " "	0.4

These results seemed to indicate a very close relationship between the two germs, which, it may be added, were possessed of very nearly the same virulence.

Similar results were obtained when I employed broth of the same composition containing 1 per cent glucose and 1 per cent lactose. The method of preparation was the same as in the previous experiments, but here the determination was made at the end of 48 hours, and a viginti-normal solution of sodium hydrate was employed in place of a decinormal, the titration being thereby accomplished with much greater ease and sureness.

TABLE IV.

	Plain Broth.	1% Glucose.	1% Lactose.
Tubes as check, uninoculated	0.5	0.6	0.5
	0.3	0.8	0.6
European germ (Kitt)	0.7	2.7	1.1
	0.7	2.4	1.0
Canadian germ	0.7	2.5	1.0
	0.6	2.7	1.1

These figures again favored the contention that the two forms are very closely related, the difference between the acid production in the different media being very slight. It is interesting to note the great increase in acid production brought about by the addition of the sugars.

But a more extensive and very carefully conducted series of observations showed that no absolute reliance can be placed upon this feature of acid production, so far as regards the micro-organism of chicken cholera.

The following table shows the results obtained by determining the acid production at the end of 24 hours in tubes of the same broth as that used in the previous series of observations, these tubes being inoculated with equal amounts of cultures of fowl cholera germs that had been subjected to different influences. A viginti-normal solution of sodium hydrate was used, and the figures represent in ccm. the amount added to neutralize 10 ccm. of broth culture.

TABLE V.

	Neutral Broth.	1% Lactose.	1% Glucose.
No. 1	0.3	0.8	0.7
	0.35	0.8	0.6
	0.3	0.9	0.7
No. 2	0.2	2.0	4.5
	0.3	2.2	4.1
	0.3	2.4	5.1
No. 3	0.3	2.0	3.9
	0.3	2.1	4.3
	0.3	2.2	4.2
No. 4	0.4	1.3	1.7
	0.4	1.2	1.3
	0.4	1.2	...
No. 5	0.4	1.2	1.5
	0.4	1.1	1.9
	0.4	...	1.5
Uninoculated tubes carried as check	0.05	0.6	0.7
	0.04	0.7	0.7
	0.04	...	...

No. 1. Bacilli of European fowl cholera passed through the fowl 10 weeks previously, and cultures since renewed every third day. This germ proved fatal to a rabbit in 6 hours, 3 cc. being inoculated intravenously.

No. 2. Bacilli from Canadian outbreak, from a broth culture taken direct from one of the St. Anne's fowls and preserved in a sealed tube for about 5 months.

No. 3. Bacilli from Canadian outbreak. Broth culture made from rabbit which had been infected through the digestive tract and had

succumbed in 36 hours. This culture had been kept in a sealed tube about 4½ months.

No. 4. Bacilli from Canadian outbreak passed through series of rabbits from rabbit which had been inoculated intravenously and had succumbed in 6 hours. This culture had been hermetically sealed for 4 months.

No. 5. Bacilli from Canadian outbreak passed through a turkey. Culture from liver renewed every third day for 3 months and 9 days.

It is to be observed from this table that there is little variation between the different series in neutral broth, but this is far from being the case in those containing sugar. A difference of 0.1 ccm. of a vigintinormal solution of the alkali added to 10 ccm. of the culture—that is, of 1 per cent in the amount of solution added—is inconsiderable, and in determinations of this nature, even when made with the greatest care, cannot be regarded as indicating any profound differences in acid production. But if this table be compared with Table 3 it will be seen that forms here giving very closely allied results to those with plain broth, namely, the European and Canadian forms, gave in other determinations wider variations, while with sugar-containing broths they gave results which were closely parallel. It is unsafe, therefore, to depend upon the apparently satisfactory results shown in this one table concerning the acid production by allied forms in neutral sugarless broth.

When we compare the results gained from lactose and glucose broths we find still greater discrepancies. So marked are they that it is immediately evident that the variations in virulence of at least one member of the group of the bacilli of hæmorrhagic septicæmia are accompanied by equally wide variations in acid production, and so far as this table may be depended upon it would seem to a certain extent that these variations are inverse, for the least virulent forms (Nos. 2 and 3) produced most acid in both lactose and glucose broths.

It is quite probable that more stable forms of bacteria may give more constant results; but it is with these unstable bacteria that we have the greatest difficulties, and are most often led to question whether we are dealing with distinct species, or merely with varieties of a common form, and so far as these observations extend it would seem evident that little or no dependence can be placed upon determinations of comparative acid or alkali production as helping to solve the problem of relationships.

CONCLUSIONS.

(*a*). The bacillus isolated by me from the outbreak of choleraic diarrhœa corresponds closely in all essential particulars with that of European chicken cholera.

(*b*). It differs, consequently, from that isolated from previous outbreaks of choleraic diarrhœa in the United States.

(*c*). A study of the acid production by this and allied bacilli would seem to show that the amount of acid produced by so variable a microbe varies greatly and is incapable of affording a further means of distinguishing between allied forms.

ADDENDUM.

ON SUNDRY SYNTHESIZED MEDIA.

In pursuing the subject of synthesized media the paper of J. J. McKenzie,* of Toronto, was taken as a working basis. The various media as given there were tried with very little success; from these others were composed, the best of which will be found below together with the results obtained. In all there were about fifty formulæ tried, including five or six taken from McKenzie's paper. In all cases the media were neutralized, phenolphthalein being used as indicator.

The first of this series was very simple, as follows:

Potassium phosphate	0.15
Ammonium chloride	0.80
Glycerine	5.00
Water	100.00

Six tubes were prepared and were inoculated with diphtheria, European fowl cholera, Canadian fowl cholera, pyocyaneus, Asiatic cholera, and the germ of the Pictou cattle disease. At the end of 24 hours the tubes inoculated with the germs of European fowl cholera and Asiatic cholera showed only slight growths, while the others showed nothing. At the end of 72 hours pyocyaneus appeared. None of the growths at this time were very vigorous. This same formula with the addition of 0.1 gramme of potassium sulphate showed in addition to the above at the end of 72 hours a growth in the tube inoculated with the germ of the Pictou cattle disease. The growths in this mixture were not vigorous. The next medium which proved itself worthy of passing notice was as follows:

Potassium phosphate	0.4
Potassium nitrate	0.8
Asparagin	0.8
Lactose	0.5
Glucose	0.5
Water	200.0

* *Journal of the American Public Health Association*, October, 1895.

This was inoculated with the same germs as the previous tubes, save that a putrefactive form was substituted for the Asiatic cholera. In 15 hours all show a slight growth, while that of pyocyaneus is quite vigorous. Diphtheria seems to grow fairly well after the first day and increases considerably in cloudiness.

Another medium which proved to be superior to the foregoing was as follows:

Ammonium succinate	0.8
Potassium phosphate	0.4
Potassium sulphate	0.2
Ammonium chloride	0.3
Asparagin	0.5
Lactose	0.5
Glucose	0.5
Water	200.0

Six tubes were taken and inoculated with the bacillus violaceus, pyocyaneus, coli, the germ of the Pictou cattle disease, European fowl cholera and that from the Canadian outbreak. At the end of 24 hours there is observed to be a very profuse growth in the case of the first three, being equal in all to that obtained ordinarily in broth, while that of bacillus coli is much better than the best growth obtainable in the same time in broth. The other three tubes remained sterile. After 48 hours pyocyaneus brought forth its characteristic color.

A still better formula was as follows:

Ammonium phosphate	0.5
Potassium nitrate	0.5
Potassium chloride	0.3
Ammonium sulphate	0.2
Asparagin	0.5
Lactose	0.5
Glucose	0.5
Water	200.0

This was inoculated with the same germs as the preceding, with a similar result excepting that violaceus was much more vigorous than in the preceding. Agar plates were made showing a good growth of the three forms above mentioned, as also of bacillus mesentericus, but there was not the character to the colonies such as is seen where broth is used.

I will mention but one more which I think is the best tried. The formula is as follows:

Ammonium phosphate	0.5
Potassium nitrate	0.5
Potassium chloride	0.3
Ammonium sulphate	0.2
Ammonium succinate	0.5
Lactose	0.5
Glucose	0.5
Water	200.0

This is practically the same as the one above save that instead of asparagin, ammonium succinate is used. Here bacillus violaceus, pyocyaneus and bacillus coli grew well. This medium was made up also with 7 per cent gelatine and gave similar results in examining water as did gelatine prepared with ordinary broth.

The following results were obtained with an agar preparation of this medium as compared with ordinary broth agar:

	Number of Colonies.	
	Broth.	Synthesized medium.
1.0 cc. water, 48 hours,	70	2
1.0 cc. water, 48 hours,	81	6
0.1 cc. water, 48 hours,	14	0
0.1 cc. water, 48 hours,	11	0
Pyocyaneus	1248	18,720
B. coli	156	260
Violaceus	No growth on either.	

The colonies were large, very distinct, and the edges were well defined. On the whole the colonies which appear are much larger than those seen on broth plates of the same age. There seems to be in this medium some element which is in such a form that the bacillus pyocyaneus has a special affinity for it and which seems to greatly increase its growth.

From the work which I have done in this line it does not seem probable that a generally serviceable medium of simple formula can be prepared, as many of the elements are required in very minute quantities. Magnesium and calcium are needed to insure success, but the great difficulty, as previous workers on this subject have noted, is to get them in such a form that they will not precipitate when subjected to the heat of the sterilizer.