The behaviour of bacteria in the digestive tract / by Allan Macfadyen, M.D. Edin.

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THE BEHAVIOUR OF BACTERIA IN THE DIGESTIVE TRACT.

By Allan Macfadyen, M.D. Edin.[1]

(*Continued from p.* 238.)

The research made by the writer is divided into two natural groups, intended to be complementary:—I. Experiments conducted *without* the body. II. Experiments conducted *within* the body.

I. Experiments conducted without the Body.

In these experiments I have made the attempt to follow out certain principles. Varieties of bacteria were employed, pathogenic and non-pathogenic, and of varying sensibility, so that the results might be of general application, and correspond more to the state of matters in the body, where we have the ingress of all forms of germs. It was also sought to copy, where possible, natural conditions. Further, germs were selected which could be readily recognised again, and a distinction was made between spore-bearing and spore-free bacteria. I made sure that all the cultures were pure in these respects. I worked only with pure cultures of individual bacteria, obtained by independent cultivation. I also distinguished carefully between the arrest of the development, and the killing of the bacteria.

It would be tedious to relate here all that pertains to the technique of the laboratory work, such as sterilisation of apparatus and material, the minutiæ of the preparation of media, &c. Suffice it to say that the most approved methods of Koch were followed out.

The *cultivating media* were gelatine and agar-agar (neutral):—

Gelatine.	Peptone.	Salt.
5 per cent.	1 per cent.	0·7 per cent.
7 „		
10 „		
Agar, 1 „		

[1] The research was carried out in the Hygienic Institute, Göttingen, and the writer would like to take this opportunity of acknowledging the helpfulness and rare kindness of its Director, Professor Carl Flügge.

The *fluids* for general use were sterilised distilled water, sterilised salt solution (0·6 per cent.).

The *colouring agent* was an alkaline solution of methylene blue, renewed every fortnight.

Cover-glass microscopic preparations were made as required. The mounting medium was Canada balsam.

The microscope used was a Winkel (Göttingen), with homogeneous oil immersion ($\frac{1}{14}$).

List of Bacteria employed in form of Pure Cultures.—All germs have well-marked characteristics, soon learned, which enable us to distinguish them as required:—

1. *Staphylococcus pyogenes Aureus* (Rosenbach, "*Mikroorganism bei den Wundinfectionskrankheiten*," Wiesbaden, 1884).
2. *Micrococcus prodigiosus.*
3. *Saprophytic bacillus.*—I isolated this from decomposing meat infusion. It liquefies gelatine, with development of green colour and characteristic stink. On plates, liquefies gelatine in three to four days, at 15° C.
4. *Bacillus pyocyaneus* (blue pus) (Gessard, "*De la pyocyanie et de son microbe*," 1882).
5. *Micrococcus tetragenus* (Koch and Gaffky, *Mittheil. a. d. Kais. Gesund Amt.*, Band ii. p. 42).
6. *Septicæmia of Rabbits.*—These bacilli colour more strongly at the poles than in the middle (Koch, *Mittheilungen aus dem Kaiser Gesundheitsamt*, vol. i. p. 94).
7. *Anthrax bacilli.*—Spore-free, by cultivating very slowly at a temperature not exceeding 14° C., and not under 12° C. (Koch, *Mittheil. a. d. Kais. Ges. Amt.*, vol. i.).
8. *Bacilli of typhoid fever* (spore-free), cultivated at 15° C. (Eberth, Gaffky, *Mittheil. a. d. Kais. Ges. Amt.*, vol. ii.).
9. Koch's *Comma bacillus of Cholera Asiatica* (Koch, *Berliner Klinische Wochenschrift*, 1884, Nos. 31, 32 33A).
10. Finkler and Prior's *Spirillus* (*Centralblatt für Allgemein. Gesund. Pflege.*, vol. i. pts. 5 and 6).

1. *The Gastric Juice.*

The medium employed was gelatine. At first I used gelatine tube cultures, to which had been added the factors of the gastric juice in the required strength; but I abandoned this, and in these researches, and in all subsequent experiments, made use of gelatine plates.

In using gelatine plates, the germs are added to the liquefied gelatine, thoroughly mixed up, and the whole poured out on the glass plate. On stiffening, the isolated colonies are caught up and fixed in different portions of the gelatine, and remain under the intimate action of the mixture.

An important procedure was the contemporaneous making of control plates. The germs in identical quantities were taken, introduced into the same amount of gelatine (pure), and the whole also poured out on glass plates. Thus, for purposes of comparison, we had side by side a natural growth of the germ, and its growth as determined in the experimental medium.

Method of Preparing the Germs.

Sterilised distilled water (10 c.c.) was introduced into a sterilised test-tube. A fresh, pure culture of the organism was then taken, and added to the water till a faintly cloudy mixture was obtained. A sterilised glass pipette was filled with this bacterial fluid, and two drops of the same introduced into 5 c.c. of sterilised distilled water in another tube, which was carefully plugged with cotton-wool. From this two drops were again taken, and introduced into the various gelatine media experimented with, as well as into the control gelatine; then well shaken up, and poured out on the glass plates. As the result of experience, I found that this gave a full control plate, with the germs fairly divided up in the mixture.

This method was used in all subsequent experiments made with gastric juice, bile, &c.

Pepsin.—I was able to heat it up to 100° C., and so to sterilise, when dry, without its losing its properties.

Hydrochloric Acid, of sp. gr. 1·1982, was taken, and diluted. The strength then determined by titration with silver nitrate. The above then added to the gelatine in the necessary quantity. The concentration was always made on 100 c.c. of gelatine at least.

The experiments were made with—(*A*) Pepsin alone; (*B*) HCl.; (*C*) Pepsin + HCl.

A. *Pepsin.*

The mixture with gelatine was inoculated with six representative

germs. Control plates were also made, according to the method described above.

The Query.—Has the pepsin itself any action on micro-organisms, and if so, of what nature? Does it arrest their development?

Temperature 15° C.	0·2%	0·3%	0·5%	1%	2%	Remarks.
1. Staphyl. aureus, .	+	+	+	+	+	The + indicates that the germs developed, and to same degree as on the Control Plates.
2. Typhoid bacillus, .	+	+	+	+	+	
3. Micro. tetragenus, .	+	+	+	+	+	
4. Anthrax bacillus, .	+	+	+	+	+	
5. Finkler and Prior, .	+	+	+	+	+	
6. Saprophytic bacilli, .	+	+	+	+	+	

Conclusion.—No hindrance of development by the pepsin when acting alone, and, *à fortiori*, no killing of the bacilli.

B. *Hydrochloric Acid.*

Method.—Here I began to imitate the natural state of things. The question of importance is, Does the hydrochloric acid kill the germs? The experiments were limited to the average time of a digestive act (four hours). They were conducted at the body temperature (37° C.). After this lapse of time, at this temperature, I sought to find if the germs were dead or not, *i.e.*, capable of growing again when removed to a suitable medium.

Gelatine + hydrochloric acid (0·05 to 0·5 per cent.). Two drops of the bacterial fluid added to each of the various concentrations; then placed in the incubator at 37° C. for four hours. At the end of this time, a quantity removed with a sterilised pipette from each of the tubes, and of this one drop, and five drops, introduced into pure gelatine, so as to give a concentrated and a dilute mixture. Well mixed up, and poured on the plates, and kept under observation at 15° C. Also the control experiments were conducted in exactly the same manner. The control plates would likewise indicate if the bacteria which I had used were really in a vital and normal condition.

The Query.—Is the hydrochloric acid the actual antiseptic factor in the gastric juice, and, if so, does it act in natural quantities so powerfully as to kill the bacteria which find their way into the stomach, and so prevent their passage in a living state into the intestine?

The boundary was taken as lying between the last plate to show any colonies, and the one above it, which had none. The diluted and concentrated plates only showed a difference in the number of colonies, and did not make any difference in the boundaries indicated in the table. The + sign indicates where any colonies were to be

seen; the 0 indicates empty plates. All the control plates developed in normal fashion, and were full of colonies. They were observed with a weak magnifying power of the microscope.

Hydrochloric Acid.

Method Described Above.	0·05%	0·1%	0·2%	0·3%	0·4%	0·5%	Remarks.
1. Staphyl. aureus, .	+	+	+	+	0	0	
2. Typhoid bacilli, .	+	+	+	+	0	0	
3. Micro. tetragenus, .	+	+	+	0	0	0	These boun-
4. Anthrax bacilli, .	+	+	0	0	0	0	daries found
5. Septicæmia of rabbits,	+	+	0	0	0	0	as results of
6. Koch's comma bac.,	+	0	0	0	0	0	six series of
7. Finkler and Prior, .	+	0	0	0	0	0	experiments.
8. Saprophytic bac., .	+	+	+	0	0	0	
9. M. prodigiosus . .	+	0	0	0	0	0	

Conclusions.—The hydrochloric acid is the active germicide in the gastric juice, but the action is much less powerful than had hitherto been supposed. We find certain forms very resistant, though placed under circumstances the most favourable for the action of the acid. Thus we find that, with an imitation of natural conditions, certain germs are still able to survive. No boundary can be fixed, but each germ must be examined on its merits. Staphylococcus aureus, and typhoid bacilli are very resistant to proportions higher than we would expect to find in the normal juice. Also, micrococcus tetragenus and putrefactive bacilli are resistant. On the other hand, anthrax bacilli and rabbit septicæmia are susceptible; while Koch's comma bacilli, the Finkler and Prior, and the micrococcus prodigiosus are the most susceptible. *A priori*, we could imagine that in the body many germs would find no difficulty in passing from the stomach into the intestine in a living state.

These experiments demonstrate that the power ascribed to hydrochloric acid is not borne out in fact, and that it must be considered as limited.

C. *Pepsin + Hydrochloric Acid.*

The Query.—Does the pepsin, then, make a difference by lowering

or by raising the boundary of the action of hydrochloric acid? Does the fermentative process prepare the germs for a surer and intenser action of the hydrochloric acid, *e.g.*, by dissolving the cellulose membranes?

For this experiment I selected typhoid bacilli (spore-free), and micrococcus tetragenus.

The peptonising action was tested first, and a sufficient amount of the pepsin taken to ensure a peptonising of the gelatine medium. The experiments conducted as under B. and control plates were made.

If the pepsin aided the hydrochloric acid, then one would find the boundary between the growth and failure of growth lowered. If it retarded the action of the acid, then the boundary should become higher.

C. *Pepsin + Hydrochloric Acid.*

	0·1%	0·2%	0·3%	0·4%
1. Typhoid bacilli, . . .	+	+	+	0
2. Micro. tetragenus, . .	+	+	0	0

The control plates were full of bacterial colonies, easily identified as those of the respective germs.

Conclusions.—The boundary limit of action remains the same as in Series B., and the pepsin does not help and does not hinder the action of hydrochloric acid.

Does the *amount* of pepsin make a difference in these respects? The same methods followed, and to the above four concentrations of hydrochloric acid the following proportions were respectively added of pepsin:—0·1 per cent., 0·3 per cent., 0·6 per cent., 1 per cent. Typhoid bacilli, and micrococcus tetragenus were again used. The phenomena remained the same as already observed, and the limit of action of the hydrochloric acid neither rose nor sank.

Conclusion.—The quantity of pepsin makes no difference in the action of the hydrochloric acid.

I did not feel called upon to investigate particularly the spores of micro-organisms. Hydrochloric acid (a watery 2 per cent. solution) only killed anthrax spores on the tenth day.

Further, the experiments of Koch already detailed, and of

others, have demonstrated that spores can easily withstand the action of the gastric juice.

To sum up these results:—

1. Pepsin alone does in no way hinder the development of the germs, and still less kill them.
2. The hydrochloric acid is the active factor in the gastric juice, but its influence is not such as the tone of previous research would warrant us to expect.
3. These experiments, conducted outside the body, cannot lead us to conclude that the acid places a serious barrier to the passage of germs through the stomach to the intestine in a living state.
4. While it is true that several germs are very easily killed, such as Koch's comma bacillus, rabbit septicæmia, and Finkler and Prior, representative pathogenic germs such as typhoid bacilli are very resistant; as also a typical germ of putrefaction, the saprophytic bacillus.
5. The resistance of certain of the germs is against such a strength of acid that, *à priori* we might say they could pass through a stomach containing a normal proportion of hydrochloric acid without being killed, and with much greater facility in states of lessened acidity.
6. The addition of pepsin, and the consequent peptonising action on the mixture does not aid or retard the acid in its work, so as to make any perceptible alteration towards a lower or a higher limit.
7. The quantity of pepsin added does not make any difference.
8. Spores are very resistant, and as a matter of fact survive the action of gastric juice.

2. *The Bile.*

What is the action of—

(*a*) The bile itself?

(*b*) Its potent constituents, the bile acids?

(*c*) The bile salts, and what contrast do they present in their action to the bile acids?

(*d*) The bile acids in the presence of other acids?

Will one be justified in concluding for a strong antiseptic action in one or any case?

It will be sufficient to show that there is an arrest of development in order to prove the rôle the bile is assigned in the digestive tract. The following experiments are arranged to investigate germs as arrested in their development:—

(*a*) *The Bile alone.—Query:* Has it an antiseptic action? Fresh ox bile was used both of neutral and weakly alkaline reaction. It was sterilised, and mixed with 7 per cent. gelatine in the proportions of 2 per cent., 5 per cent., and 10 per cent. Infected with germs according to usual methods, and therefrom plates, and also control gelatine plates. Temperature of 15° C. When control plates had grown, the bile plates were examined.

Following organisms tested:—

1. Staphylococcus aureus.
2. Typhoid bacilli.
3. Micrococcus tetragenus.
4. Anthrax bacilli.
5. Saprophytic bacilli.
6. Koch's comma bacilli.
7. Finkler and Prior's spirillus.
8. Micrococcus prodigiosus.

Results.—All grew as well as on the control plates, even with addition of 10 per cent. of bile.

Conclusions.—The bile itself, in alkaline or neutral state, in no way arrests the development of micro-organisms.

(*b*) *Bile Acids.*—Glycocholic and taurocholic acids. *Query:* Are they antiseptics with the power ascribed to them, and in the sense of the word that carbolic acid is? Do they have a potent action on pathogenic and non-pathogenic germs, or, at least, a potent control over putrefactive processes, so that an important rôle can be assigned to them in one or other respect in the intestinal canal?

I sought here not to use putrefactive germs merely, but to have a variety, pathogenic and non-pathogenic, varying in nature and in susceptibility.

I introduced two new germs into the experiments.

1. Proteus vulgaris (Hauser, "*Über Fäulniss Bacterien*," Vogel Leipzig, 1885). This is a putrefactive bacillus which rapidly liquefies the gelatine, and produces a very fœtid smell. Hauser has assigned it a rôle in septicæmic processes.

2. Emmerich's bacillus (Emmerich, *Archiv für Hygiene*, vol. ii., 1885). Emmerich believes, or believed it to be, the germ of cholera Asiatica. It is reputed to be very resistant.

In these researches we must try to preserve natural conditions, and not to experiment with amounts of the acids which could hardly be present in the intestine at one time. It will be useful to try to form a mental picture of what occurs in the intestine. What influence has the acid chyme on the bile when discharged into the duodenum?

We must imagine that the bile acid salts cease to exist when they enter the intestine, and that there we have the free acids. In so far as the acids of the chyme are stronger than the bile acids, in so far will the latter be set free, or, at least, an equipoise will be established alongside of the presence of free bile acids. At the same time, no acid is destroyed or disappears, but only hydrochloric or lactic acid is replaced by the equivalent bile acids, or one may say the titrimetric acid quantity remains equal. Maly states that in so far as the intestinal contents react acid, so far must free bile acids be present.

In human bile glycocholic is much in excess of taurocholic acid. In the dog only taurocholic acid is present.

The taurocholic acid was easily soluble in water, but with glycocholic acid I could only obtain as the strongest concentration, 0·5 per cent. Beyond that, part remained in suspension. I only used the latter in proportions in which it was soluble.

The usual solutions of germs were made, and the same quantities taken; also control plates. The neutral gelatine was impregnated with the acids, and salts to given concentrations.

Series 1.—*Taurocholic Acid.*

	0·2%	0·5%	1%
1. Saprophytic bac., .	No D.A.	No D.A.	Delayed 2 days, then No D.A.
2. Proteus vulgaris, .	No D.A.	No D.A.	Delayed 2 days, then No D.A.
3. Blue pus bac., . .	No D.A.	D.A.	D.A.
4. Emmerich's bac., . .	No D A.	No D.A.	D.A.
5. Typhoid bac., . .	D.A.	D.A.	D.A.
6. M. tetragenus, . .	D.A.	D.A.	D.A.
7. Koch's comma bac., .	D.A.	D.A.	D.A.
8. Staph. aureus, . .	D.A.	D.A.	D.A.

D.A. = Arrest of Development.

Series 2.—*Glycocholic Acid.*

	0·2%	0·5%	
1. Saprophytic bac., .	No D.A.	No D.A.	
2. Proteus vulgaris, .	No D.A.	No D.A.	
3. Blue pus bac., . .	No D.A.	Partial D.A.	
4. Emmerich's bac., . .	No D.A.	No D.A.	
5. Typhoid bac., . .	No D.A.	D.A.	
6. M. tetragenus, . .	D.A.	D.A.	
7. Koch's comma bac., .	D.A.	D.A.	
8. Staph. aureus, . .	No D.A.	D.A.	

D.A. = Arrest of Development.

Series 3.—Continued Experiments for those Germs where the upper and lower limits of developmental arrest had not been reached.

Taurocholic Acid.

	0·05%	0·1%	2%	
1. Saprophytic bac., .	No D.A.	No D.A.	D.A.	
2. Proteus vulgaris, . .	No D.A.	No D.A.	D.A.	
3. Typhus bac., . . .	No D.A.	No D.A.	...	
4. M. tetragenus, . .	No D.A.	D.A.	...	
5. Koch's comma bac., .	No D.A.	D.A.	...	
6. Staph. aureus, . .	No D.A.	D.A.	...	

Glycocholic Acid.

	0·1%	0·2%
1. M. tetragenus, . . .	No D.A.	D.A.
2. Koch's comma bacillus, .	,,	,,

Remarks.—As to Series 1, it must be at once noted that a striking and important contrast manifests itself here—the contrast between the action of the taurocholic acid upon the putrefactive and the pathogenic germs. The putrefactive displayed a surprising resistance to the acid, while the pathogenic succumbed more readily. In the case of the saprophytic bacilli, and the proteus vulgaris there was no arrest of development with 0·5 per cent. of taurocholic acid, while even 1 per cent. only delayed the appearance of the germs two days. The blue pus bacilli were arrested by 0·5 per cent. The Emmerich bacillus proved itself resistant, 1 per cent. being required for the arrest of its growth. With 0·2 per cent. the developmental arrest was complete for typhoid bacilli, m. tetragenus, Koch's comma bacillus, and staphylococcus aureus.

Series 2. The same distinction manifests itself with glycocholic acid, though obviously the effect is less potent than with taurocholic acid, as with 0·2 per cent. typhoid bacilli are still able to grow as well as staphylococcus aureus. The blue pus bacilli also suffer no complete arrest with 0·5 per cent. of the acid. The Emmerich bacillus again proves itself resistant. The

most susceptible are micrococcus tetragenus, and Koch's comma bacillus.

Series 3. It required the strong concentration of 2 per cent. of taurocholic acid to arrest the development of the putrefactive bacilli and proteus vulgaris.

For the pathogenic bacteria the arrest may be said to cease between 0·05 per cent. and 0·1 per cent. of taurocholic acid.

(*c*) *Bile Salts.*—Employed taurocholate of soda, which is very soluble, and the usual methods as already described.

Taurocholate of Soda.

	0·2%	0·5%	1%	2%	5%
1. Saprophytic bac.,	No D.A.	No D.A.	No D.A.	No D.A.	D.A.
2. Proteus vulgaris,	,,	,,	,,	,,	,,
3. Blue pus,	,,	,,	,,	,,	,,
4. Emmerich's bac.,	,,	,,	,,	,,	,,
5. Typhoid bac.,	,,	,,	D.A.		
6. M. tetragenus,	,,	D.A.			
7. Comma bac.,	,,	,,			
8. Staph. aureus,	,,	,,			

Remarks.—This salt practically does not arrest the development of putrefactive germs, while its influence over the pathogenic is weak. With 0·2 per cent. no arrest of development. First showed its action with 0·5 per cent., but at times could only assert itself in the concentration of 5 per cent.

To sum up these researches:—

Have the bile acids a potent antiseptic action? We find that the development of pathogenic germs can be arrested by taurocholic acid in the dilution of 0·1 per cent., and by glycocholic acid, 0·2 per cent. solution. But it required 2 per cent. of taurocholic acid to arrest the putrefactive process. The glycocholic acid is of much weaker power. The taurocholic acid is the potent factor. The bile salts do not practically come into the question.

These experiments reverse the relationships usually held as established, inasmuch as the control exercised by the bile acids is not over the germs of putrefaction, but rather over the germs of a pathogenic nature, *i.e.*, if it can be called a control.

Thus one cannot ascribe to the bile acids the *rôle* assigned to them by Maly and Emich, with respect to the phenomena of putrefaction. We cannot call them antiseptics worthy to rank with salicylic and carbolic acids.

(*d*) *Bile in Presence of other Acids.—The Query:* Does the bile, when acid in reaction, exhibit a marked antiseptic power? are Lindberger's results to be accepted as proving this?

Acids were selected which could be present in the intestinal canal, *e.g.*, lactic, butyric, &c.

Fresh neutral bile was used, also alkaline, and the concentrations were made in 10 per cent. gelatine. Proportions were taken which could exist in the intestinal canal, and which, if Lindberger's experiments were valid, would confirm his views.

$$\text{This mixture} = \begin{cases} \text{Gelatine, 10 per cent.} \\ \text{Bile, 5 per cent.} \\ \text{The acid, 0·05 per cent.} \end{cases}$$

The acids were taken in proportion to their specific weights. Also representative germs taken.

Temperature 15° C.	Acetic Acid. 0·05% Bile 5%	Butyric Acid. 0·05% Bile 5%	Lactic Acid. 0·05% Bile 5%
1. Saprophytic bac., . . .	No D.A.	No D.A.	No D.A.
2. Blue pus bac., . . .	,,	,,	,,
3. Typhoid bac., . . .	,,	,,	,,
4. M. tetragenus, . . .	,,	,,	,,
5. Comma bac., . . .	,,	,,	,,

Results.—No arrest of development in the bile of acid reaction. Indeed, the bacteria grew as well, if not better, on the bile plates, than on the control plates.

Hydrochloric acid 0·05 per cent., and 5 per cent. bile in 10 per cent. gelatine, gave identical results.

According to Lindberger, if we found, for example, that the acid alone, in the proportion of 0·1 per cent., arrested the development of the germ, then a smaller quantity, 0·05 per cent., in presence of bile, would have the same effect, presumably by setting free the still more potent bile acids. But the above experiments, and some later to be detailed, show that this is not the case.

The boundary with the organic acids was not lowered.

Conclusion.—The bile, in the presence of the organic acids

in proportions which, according to Lindberger, should be potent, does not arrest the development of the putrefactive bacilli or the pathogenic germs.

A further query arose :—

Is the boundary, then, raised higher when bile is set to the organic acids? *i.e.*, are the germs enabled to resist a stronger concentration of the acids?

The experiments again conducted in the usual manner, and at a temperature of 15° C. Proportion of acids 0·1 and 0·2 per cent.

I may state that these results are the same as those obtained with the same organic acids without addition of bile, as will be seen from a later series of experiments.

	Acetic Acid.		Butyric Acid.		Lactic Acid.	
	0·1%	0·2%	0·1%	0·2%	0·1%	0·2%
1. Saprophytic bac., . .	D.A.	D.A.	D.A.	D.A.	No D.A.	D.A.
2. Blue pus bac., . . .	,,	,,	,,	,,	,,	,,
3. Typhoid bac., . . .	,,	,,	,,	,,	,,	,,
4. M. tetragenus, . . .	,,	,,	,,	,,	D.A.	,,
5. Comma bac., . . .	,,	,,	,,	,,	,,	,,

Conclusion.—The bile makes no difference in the percentage amount of the organic acids required to arrest the development of bacteria, in the way of raising the limits above those found potent for the acids acting alone.

The series of experiments with the organic acids, under the heading, "Some other factors" (p. 426), will show that Lindberger's results are, in all probability, to be explained by the powerful action of the organic acids he used on bacteria.

To sum up :—

1. The bile itself plays no *rôle*, either in alkaline or neutral reaction, as an antiseptic.
2. The bile salts also have no power.
3. The bile acids are not antiseptics, as Maly and Emich have stated, of such power as to warrant the conclusion that they have a strong control over the phenomena of putrefaction. While the taurocholic acid is the potent factor, its effect is only moderate, and is exercised, not on the putrefactive, but on the more purely pathogenic organisms.

4. The addition of the organic acids to the bile makes no difference in the phenomena observed.
5. The potency of the organic acids themselves is sufficient to account for the phenomena Lindberger observed on their addition to the bile.

3. *Some other Factors.*

(*a*) *The Acids of the Chyme.—Query:* Do the organic acids of the digestive tract play any *rôle* in arresting the development of bacteria, and if so, does that action explain Lindberger's results when he added them to the neutral or alkaline bile?

Again the usual methods were adopted. Temperature 15° C. The acids were added to 5 per cent. gelatine in percentages according to weight, viz., acetic, lactic, and butyric acid.

The results obtained with these acids in the proportions of 0·5 per cent. and 1 per cent. respectively, I shall not tabulate, as in these proportions the acids arrested completely the growth of the bacteria. The succeeding experiments were made with strengths 0·05 per cent. to 0·2 per cent.

	Acetic Acid.			Butyric Acid.			Lactic Acid.		
	0·05%	0·1%	0·2%	0·05%	0·1%	0·2%	0·05%	0·1	0·2
1. Saprophytic bac.	No D.A.	D. A.	D.A.	No D.A.	D.A.	D.A.	No D.A.	No D.A.	D.A
2. Blue pus bac.	,,	,,	,,	,,	,,	,,	,,	,,	,,
3. Typhoid bac.	,,	,,	,,	,,	,,	,,	,,	,,	,,
4. M. tetragenus	,,	,,	,,	,,	,,	,,	,,	D.A.	,,
5 Comma bac.	Partial	,,	,,	,,	,,	,,	,,	,,	,,

Conclusions.—These organic acids have a potent effect in arresting development of putrefactive and pathogenic bacteria. Hitherto they are the most potent factors that I have found. They can be assigned an important *rôle* in the digestive tract in the way of arresting the development of the most varied forms of bacteria. The lactic acid is of weaker power than the others. Further, the potent action of these acids is sufficient to explain Lindberger's results when he added them to the bile.

The above results led me to examine a little the literature of this subject, and I found two theses which confirmed the experiments already detailed.

Hoffmann (24) investigated formic acid, but as it has poisonous effects I dismiss it with the remark that he found it to work strongly against putrefaction. Thol (25) also confines his

observations to putrefactive phenomena, and the effect on them of organic acids, such as acetic, butyric, lactic, oxalic, tartaric, citric, &c.

The concentrations were made in gelatine as nutrient substratum, but impure cultures were used, putrid meat infusion, swarming with bacteria, being added to the experimental medium. With lactic acid, Thol found that 0·1 per cent. could delay putrefactive phenomena till the 5th day; 0·1 per cent. of butyric delayed putrefaction till the 7th day; 0·1 per cent. acetic acid also delayed the phenomena till the 7th day; 0·05 per cent. formic acid delayed till the 8th day.

It will be seen that these results support the statement the writer ventures to make,—that the organic acids have strong antiseptic properties.

(*b*) *The Pancreatic Juice.*—That this alkaline fluid has no influence hardly requires to be stated. One has only to remember the selection of pancreas infusion for experiments with putrefaction, on account of its rapid and easy decomposition.

(*c*) *Intestinal Ferments.*—The phenomena which led me to examine them were the following:—There is a constant microscopical difference between the ordinary intestinal contents, and the mucous flakes of the same and of the intestinal wall. While the intestinal contents showed microscopically many and varied forms of bacteria, the flakes only presented a few and of limited kind. I found this in the intestine of the rabbit, dog, and man. Thus, for example, I could isolate under the microscope in the intestinal contents perhaps ten different kinds of bacteria, and in the mucous flakes only one or two. In the flakes they were often limited to one kind, *e.g.*, fine bacilli, and the germs seemed small and not much developed. Further these flakes on the plates liquefied the gelatine very quickly round about them. If many were on the same plate, the whole of the gelatine would liquefy and run off the plate, but without smell or putrefaction. In fact the gelatine was peptonised. The gelatine liquefied round about the flakes generally contained no bacteria. Also there was a great paucity in the growth of colonies, and when they did grow, they were often moulds. Finally, some of the bacteria seen in the flakes could not be recultivated on the usual media.

The Query: Do these intestinal flakes contain active ferments which act injuriously on the bacteria? In short, do the bacteria which are microscopically visible, and which yet fail to grow in the media, fail to do so because they are either killed, or, at any rate, enfeebled by the intestinal ferments? I took flakes from the small intestine of the rabbit, dog, and man, and put in small sterilised glass dishes. Then mixed thoroughly with a culture of the micrococcus tetragenus. Also a second series, with typhoid bacilli. Also control plates. Portions of the mixture of flakes and germs were introduced into gelatine, and poured out on plates. Both germs grew, but in slightly diminished numbers as compared with the control plates.

I was not able to make a sufficient number of experiments which would warrant a definite conclusion. The action of the intestinal ferments might be that of weakening the bacteria and restraining to a certain extent their activity. The subject is one which would repay investigation.

The experiments conducted *without* the body lead us to conclude:—

1. That the gastric juice cannot be taken as affording any efficient protection against the entrance of bacteria into the digestive tract.
2. That the bile acids have a limited action, which with respect to putrefactive phenomena is weak.
3. That the action of the intestinal ferments remains doubtful.
4. That the organic acids of the food chyme are antiseptics, and have been found to be the most potent factors in the digestive tract, having an action that is intense and versatile.

II. Experiments Conducted within the Body.

I selected for my experiments healthy dogs, as their digestion is more related to that of man. They also have a particularly active gastric juice, containing much more free hydrochloric acid than the human. Average acidity in dog is 0·3 per cent.

The idea was to arrange the circumstances so as to give the gastric juice the best possible chance of exerting an antiseptic action. Then to feed the animals with micro-organisms, and to search for them again in the intestine.

This would enable one to answer the question—Does the gastric juice kill the bacteria in the living body?

For these reasons I used dogs: produced only a slight filling of the stomach, by feeding with a small quantity of food, especially with a small quantity of lean meat, and starved them for twenty-four hours, so as to get clear primæ viæ, and a concentrated gastric juice. Also, during digestion of food given, allowed them no water, so that the germs might not pass too quickly through the stomach. After four to five hours, the animals were killed by inhalation of chloroform, under the influence of which they died in a few minutes. Instantaneous death was also produced in two, by passing a curved knife through the foramen magnum into the base of the brain. This was done to remove any objections to the results obtained in the dogs killed by means of chloroform. The section was at once made, and with sterilised instruments. All the surfaces were cleansed with bichloride of mercury (1–1000).

The following parts were double ligatured and removed:—

1. Stomach.
2. Duodenum, middle portion, 4 inches.
3. Jejunum, about the middle, 4 inches.
4. Ileum, upper part, 4 inches.
5. Large intestine, upper part, 4 inches.

These were placed in sterilised dishes with glass covers, opened at once with sterilised instruments, and contents removed into smaller dishes. When necessary, some sterilised distilled water added, but not more than a few c.cm.

By means of platinum loops, portions of the contents were taken out and introduced into the culture media—gelatine, 7–10 per cent.; agar, 1 per cent. From these, attenuated cultures made in a series of gelatine or agar tubes. Finally poured out on plates. Kept at temperature of 15° C. in case of gelatine, and at 35° C. in case of agar cultures.

Germ material was taken from pure cultures, spore-free. I used only germs, which could easily be recognised again in the plate cultures. Also a series of germs used of varied nature and susceptibility.

A. *Series of Three Experiments with Staphylococcus Aureus.*

The dogs were starved 24 hours, then the food + the germs given (to each contents of four agar tube cultures of staph. aureus). Drink-

ing water taken away, and the animals killed at the end of five hours. Four plates from each portion of intestine.

The numbers refer to the number of colonies found on the four plates.

Staphylococcus Aureus.

	Dog 1. 100 grs. lean meat. Killed after 5 hours.	Dog 2. 100 grs. meat. Killed after 5 hours.	Dog 3. Milk 100 c.c. Killed after 5 hours.
Stomach, ...	90 colonies.	109	2
Duodenum, . . .	None.	6	7
Jejunum, ...	12	3	2
Ileum (upper part), . .	150	None.	None.
Ileum (lower part), . .	None.	11	700
Large intestine (upper part),	None.	300	Countless.
Stomach reaction, . .	Acid.	Weakly acid.	Acid.

Observations.—These results are very striking. The stomach is placed under circumstances the most favourable for its action, and yet, instead of finding none of the cocci in the intestine, we find numbers, *i.e.*, they have passed through the stomach without being killed, and have proved themselves capable of re-cultivation again outside the body. Even in the stomach we find a number which have survived during five hours.

It will be noted that, once through the stomach, they have passed very quickly to the lower part of the gut. Where fluid diet (milk) was used the transit was very rapid, and staphylococci were found in countless numbers in the first portion of the large intestine.

It will be noted also that the results coincide with experiments outside the body, where the staphylococci survived the action of hydrochloric acid (0·3 per cent) during four hours.

Conclusion.—The gastric juice has placed no obstacle in the way of the passage of these germs, in a living state, into the intestine.

I now resolved in subsequent experiments to strengthen, if possible, still more the action of the stomach, *i.e.*, to give only meat, and in smaller quantity, and to observe more the time of a natural act of digestion, killing the dog after four hours.

Finally, to exclude the large intestine, and to observe only the small intestine as the important field for us.

B. *Experiment.*

This experiment was undertaken to get rid of a possible objection. It might be said that by starving the dogs I had lowered their vitality, and that the gastric juice was as a consequence weakened and abnormal. The germ used was staphylococcus aureus. The dog was starved for 24 hours, and then fed for 48 hours with 1000 grains meat. During the two days it was apportioned as follows :—

1st day.	12 midday,	125 gr.	500 grams.
	6 P.M.,	125 ,,	
	10 P.M.,	125 ,,	
2nd day.	7 A.M.,	125 ,,	
	12 midday,	125 ,,	500 grams.
	6 P.M.,	125 ,,	
	10 P.M.,	125 ,,	
3rd day.	7 A.M.,	125 ,,	

Then water taken away. At 12 midday, 15 grams lean meat + staphyl. aureus (4 cultures). At 4 P.M., killed.

Results :—

Staphylococcus Aureus. Dog 4.

1. Stomach, . . .	20 colonies.	The reaction of the stomach was strongly acid ; contained no solid remainder of food.
2. Duodenum, . . .	90 ,,	
3. Jejunum, . . .	40 ,,	
4. Ileum (upper part), .	6 ,,	
4. Ileum (lower part), .	None.	

Remarks.—The cocci were able to pass through the stomach alive, under those conditions so favourable to the action of the gastric juice.

Series C.

This series was arranged in order to test a variety of organisms, and of varying susceptibility, as will be seen in the tables of action of hydrochloric acid, under the heading "Gastric juice."

The germs used were :—

1. Comma bacillus of Koch, contents of 4 tubes—a very susceptible germ.
2. Septicæmia of rabbits, 4 fresh cultures : this germ also delicate.
3. Anthrax bacilli, and free from spores. Rabbit's liver and spleen were taken, and mixed with some meat, and given to the dog to eat. They were removed immediately after death, and were free from spores. Also, control plates were made from a portion of the spleen, and anthrax colonies developed. The dogs were starved for twenty-four hours, then fed with 50 grms. meat + the germs, and after four hours killed.

Tables of these Experiments.

	Dog 5. Comma Bac.	Dog 6. Rabbit Sept.	Dog 7. M. Tetrag.	Dog 8. Anthrax Bac.
Stomach, . .	No colonies.	None.	5 colonies.	None.
Duodenum, . .	None.	,,	4 ,,	,,
Jejunum, . .	,,	4 colonies.	4 ,,	,,
Ileum (upper part),	,,	None.	None.	,,
Ileum (lower part),	,,	,,	,,	,,
Stomach reaction, .	Strongly acid.	Acid.	Weakly acid.	Acid.

To prove, in case of rabbit septicæmia, that the colonies found in the jejunum were really those of that organism, a mouse was inoculated from them. In twelve hours it died, and in blood of heart and in spleen the characteristic bacteria were found.

Remarks.—These results are of great interest. Only two of the four germs passed the stomach alive, viz., tetragenus and rabbit septicæmia. The germs which were killed in the stomach were the comma bacilli, and the anthrax bacilli. The last two are very delicate. The stomach, then, only proved a protection against anthrax and Koch's comma bacilli, and that under the most favourable circumstances for the action of the gastric juice, while the rabbit septicæmia, a delicate germ, was able to reach the intestine alive. These results confirm the experiments conducted outside the body.

Thus we have found germs passing through the stomach alive, and reaching as far as the ileum, and beginning of the large intestine.

Series D.

I took the germs I had failed with, viz., cholera and anthrax bacilli, and arranged the experiments with them in a different form. In fact, made the conditions those least favourable to any injury of the bacteria, viz., a quick passage through the stomach, by means of a purely fluid medium.

Accordingly, two dogs starved for twenty-four hours. Then 200 c.c. water given, impregnated with cholera and anthrax bacilli respectively, four cultures of each. Then, no water, and the animals killed after four hours.

	Dog 9. Anthrax Bac.	Dog 10. Comma Bac.
Stomach, . . .	No colonies.	None.
Duodenum, . .	None.	,,
Jejunum, . . .	3 colonies.	,,
Ileum (upper part), .	None.	,
Ileum (lower) . .	,,	,,
Stomach reaction, .	Acid.	Acid.

Remarks.—Thus, we find that, given a fluid and a consequent quick passage into the intestine, a germ so delicate as the anthrax bacillus can pass through a normal stomach. This minimises still further the protective value of the stomach against the incursions of bacteria. Of all the germs of varying nature and susceptibility, I only failed with the comma bacillus of Koch; and one must remember how delicate it is, and how concentrated and strong the gastric juice of the dog was. One can readily imagine that with a weaker gastric juice and much fluid, even the comma bacilli could pass into the intestine. Some later experiments with dogs fed with water and comma bacilli, and killed within an hour, gave abundant colonies of the germ in small intestine. An important fact. It was thus my good fortune to succeed with one and all of the germs selected for the experiments, and to bring this portion of the research to a satisfactory conclusion.

These animal experiments, which may be considered as complementary to the artificial researches, confirm the latter, and justify us in saying that we have no special protection afforded us in the gastric juice. Further, given the weaker human gastric juice, with mixed solid and liquid diet, pathogenic and non-pathogenic germs will constantly be passed on alive into the intestine. Also, abnormal states, with lessened acidity, will still more favour the transit.

Finally, the germs once in the intestine will find their chief foes in the organic acids of the food.

Some Concluding and Complementary Researches.

1. Do germs pass through the normal intestinal wall? Do the germs find their way from intestine into organs and blood, and *vice versâ?*

Bearing in mind the passage of fat globules, one is led to the idea that the germs in the gut might also find their way through the intestinal wall. However, Dr Wyssokowitsch, with bacillus indicus and bacillus subtilis, obtained negative result. Indeed, we do not feel called upon to consider such a migration as likely, having regard to the epithelial lining and compact walls of the gut.

A migration can probably only occur when there is a solution of continuity.

Ribbert has stated that he has microscopically found a wandering of bacteria through the healthy epithelium in the follicles of the processus vermiformis of rabbits. In the deeper layers of the follicles, however, the bacteria seemed to have lost their vitality, and could not be traced farther.

Bizzozero has lately published similar results.

We can readily picture a constant fight as going on between the living cells of the body and the micro-organisms, in which fight the living cells usually conquer. Metschnikoff asserts that the leucocytes of the blood are special enemies of the bacteria, and that they destroy them. The bacteria also, in the normal living tissues, find mechanical hindrances to growth and a pre-assimilation of disposable nutriment.

In fact, with intact epithelium and the compact intestinal wall, we have an efficient germ filter.

2. Are those germs which microscopically we recognise in the intestinal contents, and which are not capable of recultivation, killed or enfeebled bacteria, or are they germs which do not grow, simply because the usual media and methods of research are not favourable for them? Can one increase the number of germs recultivable by other methods? Are those germs anærobic?

We have distinctly anærobic germs present in the digestive tract, *e.g.*, the clostridium butyricum, and the bacillus of malignant œdema. The latter is found in the intestine of the rabbit, and is exquisitely anærobic. Also, there are germs which, whilst most active in the presence of oxygen, still grow without air, though slower, *e.g.*, bacillus saprogenus (Rosenbach).

The following investigations were made to see if, after cultivating germs without access of oxygen, many grew, and if there still remained an important surplus:—

Method—

Gelatine, ...	5 per cent.	}
Sugar, ...	2 „	}
Agar, ...	1 „	}
Sugar, ...	2 „	}

I took respectively a loop full of contents of small intestine of dog,

and of rabbit, and put in sterilised glass dish, and added to the same 20 drops of sterilised distilled water, and stirred well up. From this, with a sterilised fine pipette, one drop—

(*a*) On cover glass for microscopical examination.
(*b*) In ordinary gelatine for cultivation by the ordinary methods.
(*c*) In sugar, gelatine, or agar, for cultivation with exclusion of air.

Thus could establish a comparison.

Used for anærobic experiments round glass dishes, with margins 2 centimetres high. The vessels filled at least $1\frac{1}{2}$ centimetre deep with the gelatine or agar, as many of the germs are delicately anærobic; *e.g.*, malignant œdema grows best at depth of 1 centimetre.

Gelatine at 15° C., agar at 35° C. Apparatus, usual hydrogen generator.

The anærobic apparatus need not be described in detail. A stout copper circular vessel, lined with zinc, and furnished with a tightly-fitting lid having two apertures with stop-cocks, was used.

The lid, by means of screws, was fastened down on a broad india-rubber band. Before use it was tested to see if air-tight, by means of a mercurial manometer.

The cultures were placed on perforated metal trays inside the apparatus, and the lid then screwed down. Hydrogen gas passed through apparatus for half an hour. Then the stop-cocks closed, and the whole placed in an incubator for four to six days, opened, and plates examined. In apparatus a culture of proteus vulgaris placed to absorb any oxygen still present.

I was able to get a number of germs to grow which were wholly absent from the plates treated in the ordinary fashion. Time did not permit an inquiry into their morphology. Many of the bacteria microscopically visible must be active, and not enfeebled, organisms. The germs still not accounted for may be considered to be bacteria enfeebled through the gastric juice, the organic acids of the food, and perhaps the intestinal secretions.

Concluding Remarks.

The researches the writer has made show that, on the whole, a slender protection against bacteria is afforded to us through the secretions of the digestive tract, and especially under natural conditions, with a great mass of nutriment, much fluid, easier gastric disturbances, and consequent lessened acidity.

We find a weakened gastric juice in fevers, anæmia, gastric catarrh, nervous states, motor disturbances, and a delay of food in the stomach, with consequent fermentation (lactic acid, butyric acid, and gases developed). In the stomach and the intestine, as the result of the action of bacteria, digestive phenomena may easily pass into putrefactive. The absorption of the products

has probably much to do with the cause of obscure general bod symptoms.

There must be a frequent passage of active bacteria throug the stomach.

Obviously a much wider protection must be given throug the impossibility of an absorption taking place. We have only to call to mind the thick and layered intestinal mucous membrane, with its epithelium acting as a filter; also the difficulty colonies have in settling on the healthy mucous membrane, and how few are found in the mucous flakes taken from the healthy gut.

Medically, this fact is of great value, pointing to the importance of the local factor in disease, especially of the intestinal tract. Given a fermentation, arising, say, in the intestine of a child—a catarrh ensues, and the colonies of bacteria can settle. Also a solution of continuity of the epithelium, or an inflammation, will allow bacteria to settle in the walls, and thence spread into the body. Pasteur found that when he fed animals with sharp substances along with anthrax, he could produce the disease very easily.

This research, then, enables one to lay more stress on the "local predisposition" as an important factor in disease.

But while the bacteria, under ordinary conditions, are not absorbed, their products can be; and the researches detailed in this paper help to make still more important the dietetic handling of disease, and how a stringent control, helped by a clear understanding of fermentative processes, should be exercised over bacteria in the digestive tract by means of drugs, &c. Infants' milk, before use, should be protected from the entrance of germs.

The bacteria can develop poisons out of the animal constituents. Some diseases would seem to be intoxications from such fermentative products. Now we can isolate the bacteria, and examine their products, and so come nearer to getting specifics against specific enemies, inasmuch as we know that the accumulation of certain metabolic products of the bacteria can directly destroy their originators. The separation of *specific* Ptomaines would greatly aid the advance of rational Therapeutics.

The researches detailed show that the organic acids of the food have a not unimportant influence over bacteria. They probably hinder the development of fermentations by killing or paralysing the germs. It seems as if they could have the power to kill out extraneous bacteria which have nothing to do with the intestinal processes, and to leave the path free for the usual fermentations. This may also explain their importance as dietetics, *e.g.*, sour milk, vinegar, pickles, mustard. Clinically, too, in abnormal fermentations, lactic or acetic acids might be given instead of hydrochloric acid.

These acids also can probably enfeeble bacteria, so that they are not capable of recultivation; and taking this in conjunction with the number of anærobic bacteria found in the gut, one is brought again face to face with the local predisposition as a great factor in disease.

Finally, these researches enable us to say, that in the gastric juice and intestinal secretions we have very little protection against the organisms which find their way into the digestive tract.

The food acids are able to paralyse the germs and hinder the development of fermentations, when these are not stopped by the products of the bacteria themselves. In the intact walls of the intestine we have an efficient barrier against the absorption of micro-organisms.