THE BACTERIA OF THE STOMACH.¹

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Two points have been constantly overlooked in the previous investigations into the growth of the organisms usually to be found in the human stomach before or after meals. The media in which they grow in this organ are generally acid, and this acid is known to exercise an inhibitory power over them. Hitherto it has been looked on as free hydrochloric acid, and the total acidity of the stomach contents has been regarded as due solely to free acid in estimating its power against micro-organisms. That any experiments, conducted by means of free HCl, and designed to show the bactericidal properties of this acid, are not applicable to the effect normally produced in the stomach by the total acidity present, is evident when the component constituents of that acidity, as shown by Hayem and Winter (¹), Ewald (²), and myself (³), are considered. Instead of the free HCl being from $\cdot 2$ to $\cdot 3$ per cent., or equal to the total acidity, it is rarely, even in the latest stages of digestion, more than $\cdot 05$ per cent., or at most $\cdot 1$ per cent. The difference between these figures indicates the combined acidity,—the amount of hydrochloric acid combined with proteids, the proteid-hydrochlorides as I have elsewhere (⁴) called them. These facts, then, modify the question very considerably. Another point, however, remains. If we take Macfadyen's experiments (⁵) as a type, we find that he estimated the maximum antiseptic power of hydrochloric acid by means of gelatine plates, adding to their contents known quantities of HCl. What happened? Some of the added acid combined with the proteids of the nutrient media, proteid-hydrochlorides were formed, and the deductions from the experiments were falsified. Again the acid was added in the maximum strength at the beginning of the experiments, and did not vary during them, a condition at variance with normal digestion. Macfadyen used well-known organisms—pathogenic and non-pathogenic—in his investigations, none of which, however, could be

¹ Part of a Thesis for the degree of Doctor of Medicine, for which a gold medal was awarded by the University of Edinburgh.

said to normally inhabit the stomach. I have used, for the most part, organisms which were grown from the gastric contents of patients.

It was not my intention to endeavour to isolate as many organisms as possible. That has been already done. Nor is it my purpose to go fully into the literature of the subject, a good *résumé* of which will be found in Macfadyen's paper above referred to. Suffice it to say, that this observer experimented with the following organisms:—

1. *Staphylococcus pyogenes aureus*.
2. *Micrococcus prodigiosus*.
3. Saprophytic bacillus.
4. *Bacillus pyocyaneus*.
5. *Micrococcus tetragenus*.
6. *Bacillus* of the septicæmia of rabbits.
7. Anthrax bacillus.
8. Typhoid bacillus, free from spores.
9. Koch's comma bacillus.
10. Finkler and Prior's spirillum.

These germs were grown on gelatine plates, the gelatine of which was rendered acid by the addition of HCl, or to which pepsine had been added. Pepsine alone had no effect on the organisms. Hydrochloric acid, either alone or along with pepsine, hindered the growth of all the organisms. The presence of the pepsine did not modify the action of the acid.

Staphylococcus pyogenes aureus was killed by .3 per cent. HCl.

Typhoid bacillus by .3 per cent. HCl.

Micrococcus prodigiosus and Finkler's spirillum by .05 per cent.

Micrococcus tetragenus and the saprophytic bacilli by .2 per cent.

Anthrax bacillus by .1 per cent.; and Koch's comma bacillus by .05 per cent.

On further testing the antiseptic action of the fatty acids on the same germs he found that, as a general rule, the acids of this series were more powerful bactericides than HCl.¹

In another research the same author (6) identified a number of organisms in the small intestine, the numbers and forms of which varied after different foods. These organisms must therefore have, shortly before, run the gauntlet of the gastric juice, and a consideration of them is pertinent to the present subject.

The following were the organisms observed:—

1. After a meat diet—

- (a) A liquefying bacillus (*B. liquefaciens ilei*).
- (b) A bacillus-like bacillus (or bacterium)—*B. coli communis*.
- (c) An oval non-liquefying bacillus.
(These grew in large numbers.)
- (d) An ellipsoidal bacillus.
- (e) A large plump bacillus.

¹ Duclaux has also pointed this out, and quite recently, that one of these fatty acids (formic) is a very powerful antiseptic. *Annales de l'Institut Pasteur*, tome vi., p. 593.

- (f) A non-liquefying streptococcus.
 - (g) Yeasts.
 - (h) Moulds.
- (Not so numerous.)
- (i) A short, thick bacillus, with rounded ends (*B. lactis aërogenes*).
 - (k) A short red bacillus, with flattened ends (*B. ilei*).
- (These last two on another occasion.)
2. After a carbohydrate diet—
- (a) A bacillus like the bacillus coli communis.
 - (b) A non-liquefying diplococcus.
 - (c) A smaller non-liquefying diplococcus.
- (These commonly occurred.)
- (d) A liquefying streptococcus (*Streptococcus liquefaciens ilei*).
 - (e) A slender rod-shaped bacillus.
 - (f) A large chain bacillus.
 - (g) Yeasts.
- (These less often.)

The number of micro-organisms, therefore, grown from the contents of the small intestine was very considerable.

Among the other investigators of this subject, who have cultivated micro-organisms from the stomach contents, may be mentioned Falkenheim⁽⁷⁾, who especially devoted his attention to the *Sarcina ventriculi*, Richter⁽⁸⁾, Abelous⁽⁹⁾, who obtained sixteen different varieties, Capitan and Morau⁽¹⁰⁾, Falk⁽¹¹⁾, Naunyn⁽¹²⁾, and Bienstock⁽¹³⁾.

Others have experimented on the antiseptic power of the contents of the stomach, notably Frank⁽¹⁴⁾, Miller⁽¹⁵⁾, Maly and Emich⁽¹⁶⁾, Lindberger⁽¹⁷⁾, Thol⁽¹⁸⁾, and Straus and Wurtz⁽¹⁹⁾.

Mere mention of these names must here suffice.

OBJECTS OF THE RESEARCH.

For the sake of clearness the objects of the research may be tabulated:—

1. To cultivate and identify organisms from the stomachs of patients.
2. To ascertain their products on various media, especially with regard to their acid-producing power.
3. To estimate the effect of hydrochloric acid added to them in a similar manner to that in which it is added in life, and, as far as possible, under the same conditions.
4. To deduce from the facts observed any points bearing on the etiology of dyspepsia, and the treatment of that group of diseases.

Dr. Claud Muirhead very kindly allowed me to obtain specimens of the gastric contents of patients, who were in his ward in the Edinburgh Royal Infirmary, suffering from various ailments. In each case the nature of the disease has been recorded, and its more salient features noted. The fluid obtained was also carefully analysed, and the results tabulated.

During the investigation of the course of the different acidities in normal digestion, I obtained figures closely corresponding to those

observed in the dialyser experiments which follow. The gradual dialysis of the acid from without into the tube, the combination of part of that acid with the contained proteids, and the non-appearance of free HCl until the half hour or the hour, as the case may be, may be regarded as almost identical with the conditions during life. The differences are, the want of rhythmical movement in the contents, and the absence of the "living principle." Sheridan Lea⁽²⁰⁾, in his research on digestion in dialysing tubes, regarded the increase in the production of peptones over a similar digestive act in a flask to removal of peptones as they were formed. The gradual dialysis of the free acid into, and of the combined acid out of, the tube is more probably the cause of the increase.

METHODS.

A method had now to be found by which micro-organisms from the gastric contents could be cultivated in a manner free from the objections urged above. This was attained when parchment sausage tubes were used for digestion by means of dialysis. A loop of this parchment tube was suspended in a beaker, furnished with a wooden lid having two circular holes drilled in it, sufficiently large to allow the ends of the tube to be passed through. The beaker was filled with solutions of HCl in water, of varying strengths. The tube was filled with sterilised bouillon, or other nutrient medium, inoculated with the organism to be tested, the ends drawn through the wooden cover and tied together, above. When the lid was put on, the parchment tube dipped into the acid solution. The parchment tube before use was washed with boiling water both inside and out, and then rinsed with absolute alcohol. The ordinary bacteriological precautions were taken in sterilising the bouillon, gelatine, platinum needles, etc.

During the experiments the beaker with its contents was placed in an incubator, heated by hot water, and at from 38° to 40° C.

From time to time gelatine tubes were inoculated by means of a platinum needle from the contents of the parchment dialyser, and some of the contents removed by means of a sterilised pipette. Gelatine plates were made from the tubes. The portion removed served for quantitative estimation of the acids.

The organisms experimented on were obtained from the stomach in the following manner:—An ordinary soft india-rubber stomach tube, washed first with boiled water then with alcohol, was used. After passage of the tube the first 20 or 30 cubic centimetres of the stomach contents were allowed to escape, then a small quantity was caught in a sterilised test-tube, which was promptly re-plugged with cotton wool. From these gelatine plates were made, and the organisms were isolated and grown pure. These pure cultivations were used in the digestion experiments.

As is usual for the purposes of identification, organisms, obtained

from the stomach contents, were grown on different media. These growths were used for analysis, especially of the acidity, after their characters had been noted. The form of acid present, and the presence or absence of peptones and alcohols, were also investigated.

In most of the experiments pepsine was added to the contents of the tube, and very considerable difficulty was experienced in sterilising it satisfactorily. The dry pepsine used at first was full of a large form of *Bacillus subtilis*, indeed it gave almost a pure culture of this organism. Drying over sulphuric acid and then heating up to nearly 60° C. for several days sufficed to sterilise it without destroying its digestive powers; but better results were obtained from the use of an acid glycerine of pepsine, prepared by Duncan & Flockhart of Edinburgh, a very small quantity of which was sufficient for the digestion of the amount of proteid usually placed in the parchment tube.

In analysing the fluid, both from the tube and from the beaker, the following tests were employed:—

The total acidity was estimated in the usual manner by titration with a decinormal solution of soda, using phenolphthalein as indicator.

The acidity combined with proteids was obtained by titrating, in a similar manner, a known amount of the fluid, after drying at 100° C. for some hours, and redissolving in distilled water.

The free acid present was equal, of course, to the difference between these two results.

The presence of free HCl was shown by Günzberg's vanillin-phloroglucin, or by Liebermann's test.

Uffelmann's carbolo-ferric test sufficed to indicate the presence of lactic, acetic, and butyric acids. If, however, the result of this test was in any way doubtful, when applied to the detection of the last two acids of the series, formation of their ethers, by boiling with caustic potash and absolute alcohol, served as a means to check the result.

Lieben's iodoform test enabled one to detect the presence of alcohol, and Fehling's solution that of peptones and sugars.

It is not within the scope of this paper to defend the procedure adopted in estimating the free and combined acidity. I have elsewhere discussed this point in part ⁽²¹⁾, and I hope soon to have an opportunity of explaining and defending it more fully.

It is not, perhaps, necessary to enter into the particulars of the ordinary bacteriological routine; they are now so well known. It will be well, however, to describe one method which was used in cultivating organisms from the gastric contents. The bacteria, of course, in this viscus, live at a temperature of from 99° to 100° Fahr.; and it appeared to me probable that a greater number would grow at that temperature than could flourish in a temperature below the melting point of gelatine. Tube plates of agar-agar were therefore used; these tubes were heated at first to boiling point to melt the agar-agar, then cooled to 100° Fahr., and while still fluid were inoculated. Rapid cooling in cold water, while

revolving in a horizontal position, caused a thin layer of the nutrient material to be deposited on the glass. These tubes could now be kept at a 100° Fahr. without further danger of their contents liquefying. By this means several organisms were cultivated, which in all probability would have perished at the ordinary temperature. This method has been described independently by Messrs. Hayer and White (²²).

The anærobic cultures were grown in bouillon under a stratum of sterilised olive oil.

The bouillon used throughout the investigation was made by the addition of the juice extracted from 500 grammes of lean beef to 1 litre of water. Ten grammes of powdered beef peptone, and 5 grammes of HCl were then added, and the solution filtered. The reaction of the bouillon was always neutral.

The agar-agar was made up of a litre of water, the juice of 500 grammes of lean beef, 10 grammes of peptones, 5 of NaCl and 10 grammes of agar-agar.

One hundred grammes of pure gelatine added to 1 litre of bouillon formed the ordinary gelatine medium used; it was slightly alkaline in reaction.

All acidities are calculated, throughout the paper, in terms of HCl.

MICRO-ORGANISMS GROWN FROM THE STOMACH CONTENTS.

The following are the 24 micro-organisms which were obtained from the contents of the stomachs of different individuals, with their characters and source:—

1. *Bacillus coli communis*.—This was either the bacillus itself, or the form indistinguishable from it described by Macfadyen. It was grown from the stomach contents of a patient suffering from dilatation of that organ, which reached from high up in the left axillary region to midway between the pubis and umbilicus. He suffered from flatulence, pain an hour and a half after eating, and constipation. His symptoms were entirely relieved by lavage. The organisms were short bacilli, with sharp ends of from 1–4 μ in length, $\cdot 4 \mu$ in thickness, and formed small white circular colonies in gelatine plates. In “stroke” cultivation, a slightly yellow transparent membrane, smooth on the surface, and with curved wavy but smooth edges; on potatoes the growth was in the form of a moist light yellow membrane; milk was coagulated. The gelatine was not liquefied, but in “stab” cultures bubbles of gas were formed along the track of the needles. The organisms were slightly motile.

The stomach contents on the occasion on which the bacillus was obtained were of $\cdot 288$ per cent. acidity, a trace of free HCl being present.

On another occasion the acidity was $\cdot 144$ per cent. with no free HCl.

2. *Sarcina ventriculi*.—Obtained from the same patient as the foregoing, absent when he came into hospital, at which time the acidity of his gastric contents was as high as $\cdot 481$ per cent.; it first appeared when, from treatment by lavage, the acidity had been somewhat reduced. The same thing was observed in two other cases of dilated stomach—with hyperacidity—from which the organism was ultimately grown.

3. *Mucor*.—One of the mucors was also grown from the same patient as the two foregoing organisms. It grew quickly on gelatine at the ordinary temperature, forming white crusts on the surface, and beautiful true-shaped coniferous forms below, like inverted spruce firs covered with snow. Gelatine not liquefied, mycelium in this medium poor. In bouillon, however, mycelium form was prominent, as also on bread paste. On potato, formed a moist growth, slightly yellow, irregular, with raised protuberances. It did not coagulate milk.

4. *Saccharomyces cerevisiæ*.—Grown from a patient suffering from simple dyspepsia, the contents of whose stomach was of $\cdot 15$ to $\cdot 18$ per cent. acidity, with both free HCl and butyric acid present. Size, from 7 to $9\ \mu$.

5. *A slender leptothrix* (from the same case as No. 4, and growing along with it).—Grew slowly on gelatine, forming under the low power diffuse colonies, under the high power aggregations of long threads $\cdot 2\ \mu$ broad when fresh, much curved, twisted, and entwined. It did not liquefy the gelatine, while in bouillon it formed rods four or five times as long as broad. It coagulated milk, grew as a slight, flat, colourless growth on potatoes, and formed no spores. Affinity for aniline stains not marked.

6. *Proteus vulgaris*.—This form was obtained from the stomach of a child, suffering from constant vomiting. The fluid received was small in amount, only slightly acid, and contained neither free HCl nor lactic acids. The organisms were $\cdot 5\ \mu$ broad, but of varying lengths, often $1\cdot 2$ to $3\cdot 5\ \mu$, when measured after mounting.

The gelatine plates showed numerous liquefying colonies—circular, whitish and turbid, brown in colour, especially towards the centre, under the low power. Under the high power short rod-shaped bacteria were observed.

The gelatine in tube cultivations was soon entirely liquefied, the fluid having a yellowish-brown colour and a brown sediment. Milk was slowly coagulated. It formed no spores, was motile, and had a disagreeable odour.

7. *A small-celled yeast* (*Saccharomyces minor*?).—Separated from the stomach contents of a woman suffering from dyspepsia, who only a short time before had partaken of bread and milk. Acidity $\cdot 324$ per cent., no free HCl or lactic acid, acetous smell. Uffelmann's test for acetic acid gave well-marked reaction.

This organism grew very similarly to the larger variety, but individuals only measured from $5\ \mu$ to $6\ \mu$ in diameter.

8. *A large leptothrix form*.—Obtained on two separate occasions along with the last-named organism. The organism measured $\cdot 75\ \mu$ in breadth when fresh. On gelatine plates white ill-defined colonies were formed, growing slowly and spreading down; no liquefaction occurred. Under the low power the colonies were seen to be of a light brownish colour with indistinct margins. Under a higher power the bacilli were seen to be long and curving, and thicker than No. 5. Perhaps the organism is identical with the large chain bacillus seen by Macfadyen in Series 2 (f) (p. 281). In bouillon shorter rods were formed, $3\ \mu$ long, $\cdot 75\ \mu$ broad, slightly motile. Milk was coagulated from the bottom, when grown anærobically on bouillon non-motile short rods slowly appeared.

9. *Bacillus subtilis* (Buchner).—Grown from some waterbrash fluid which was obtained from a woman suffering from slight dyspepsia. The fluid was acid $\cdot 081$ per cent., no free HCl, and no lactic acid; a trace of alcohol, acetic acid, and sulphocyanide of potash. Spores were observed. Individual bacilli were 5 to $6\ \mu$ long, $1\cdot 5$ to $2\ \mu$ broad, ends slightly rounded, and were motile to a small extent. Colonies on gelatine plates quickly liquefied the jelly in circular pits. Under the low power the colonies were clear at the margins, darker towards the centre, while the organisms were seen to be bacilli about three times as long as broad, and often growing in chains.

The surrounding gelatine was uncoloured, while the fluid was yellowish, and a white sediment was deposited.

The growth had an offensive odour and a marked alkaline reaction. On agar-agar it gave rise to a thick white growth with curved edges, and had a smell like Stilton cheese. On potatoes there was a light yellow moist growth, membrane-like, with curved edges, and a cheesy smell. Later, this growth became dry and wrinkled, air bubbles forming beneath it.

A soft crumbly coagulum was produced in milk.

10. *Micrococcus candicans* (from a patient suffering from cancer of the omentum, who had his stomach washed out for some local discomfort).—The contents, four hours after breakfast, were ill-digested and sour. Analysis showed—Acidity, .18 per cent. ; chlorides, .234 per cent., all combined or fixed ; no free HCl, traces of lactic and butyric acids.

This and the next organism were grown from the same case. Measured in the fresh state the organism was 1.1μ in diameter, $.8 \mu$ from a growth in agar mounted in balsam.

The micrococcus formed in gelatine small white non-liquefying colonies, which were of a yellowish colour under the low power, while under the high power the micrococci were seen to be large, and arranged either singly or in heaps. No diplococci or streptococci were seen.

In "stroke" cultivations, a thin stearin-like growth appeared, which broadened but never spread much over the surface.

The micrococcus did not cause coagulation of milk, and formed slight colourless growths on potatoes. In glucose solution there was a pronounced growth, while bouillon became slightly acid if air was admitted, no growth occurring under anærobiosis.

11. *A slender bacillus growing in light yellow colonies (Bacillus luteus liquefaciens)*.—Size of organism : length, 2.5 to 3.5μ ; breadth, $.3 \mu$, when stained with methyl-violet and mounted in Canada balsam.

On gelatine plates small yellow punctiform growths appeared, slowly liquefying the gelatine. These, under the low power, were dark yellowish-brown in colour, with blurred edges ; under the high power, long thin non-motile bacilli could be recognised.

On gelatine tubes they formed, in puncture cultivation, at first a yellow "nail-head" growth, which slowly ate down through the gelatine, forming a funnel, at the foot of which a dark orange sediment collected ; the liquefied gelatine was light yellow in colour and clear, the surrounding gelatine uncoloured. Very little smell. On agar-agar the cultivation was of a darker yellow, while bouillon became turbid, yellow, and when shaken had an appearance like watered silk. On potato a very bright yellow appeared, the bacilli being a little shorter, 2.25μ ; milk was not coagulated, but soon began to smell badly. No growth occurred in glucose or under conditions of anærobiosis.

12. *Micrococcus* (from a case of anæmia with atonic dyspepsia).—Patient had been on a diet solely of milk before extraction of the stomach contents, which were obtained three-quarters of an hour after a meal. The milk was coagulated, and had a disagreeable smell. The acidity was only .018 per cent., no free HCl or lactic acid, smell of butyric acid.

The only difference observed between this organism and No. 10 was that the growth in bouillon became markedly alkaline, and formed a tenacious sediment. The size and growth in other media were similar.

13. *Bacillus fluorescens liquefaciens* (from the same case as the last).—Length of organisms in fresh state, 2 to 3μ , often joined together, motile. Gelatine was liquefied speedily, the colonies having a dark centre and a clear periphery when examined under the low power. Puncture growths on gelatine first gave rise to a white line along the course of the needle, but the top of

this soon became funnel-shaped and filled with a greenish turbid fluid with a white deposit. The gelatine round also began to be tinged with green. The growth went on until all the gelatine had been liquefied. Agar-agar was also tinged green, the colour lasting some weeks, afterwards however being replaced by a yellow-brown.

On potato the growth was yellow, uniform, and dry, some discoloration of the potato close by. In bouillon the green colour was badly marked, while the growth was slow. Anærobically there was little or no growth.

14. *Rosa Hefe* (*Pink torula*).—This organism appeared in many cases. It is so well known that the characteristics of its growth need not be detailed here. One patient from whom it was cultivated had a dilated stomach, the contents of which were ·216 per cent. in total acidity, ·0312 per cent. in free HCl, ·162 per cent. combined HCl, ·0138 per cent. organic acids, chiefly butyric. In others the acidities were less.

15. *Bacillus* (*Weisser*?) (from the same case as detailed above, but on a different occasion, when the acidities were much greater).—Total acidity, ·432 per cent.; free acidity, ·144 per cent.; combined acidity, ·288 per cent.; free HCl present; some lactic acid.

Length of bacillus, 4 to 5 μ ; breadth, 1 μ , in fresh state, many curved. When stained with methyl-violet showed, in some cases, dumb-bell forms.

This organism formed white round non-liquefying colonies on gelatine, which were light brown, with, under the low power, indented and marked edges. Under the high power somewhat large curved motile bacilli could be distinguished, often growing in strings. On agar-agar a large white, fleshy growth appeared, at first like milk drops, which soon coalesced. Milk was slightly coagulated from the top; anærobically very little growth.

16. *Bacillus*?—This organism is of interest, as it was only after three attempts had been made from the same patient that any growth appeared on the plates.

The patient had a dilated stomach, with hyperacidity. On the first day, 4½ hours after food, 96 ounces of fluid were withdrawn from his stomach, the analysis of which was as follows:—

Total acidity, . . .	·414 per cent.	Free HCl, . . .	·0624 per cent.
Combined acidity, . .	·324 „	Total chlorides, . .	·4992 „
Free acidity, . . .	·09 „	Combined chlorides, .	} ·4368 „
Free organic acids, . .	·0276 „	Fixed chlorides, . .	

No growth.

Two days after the analysis showed:—

Total acidity, . . .	·324 per cent.	Free HCl, . . .	·018 per cent.
Combined acidity, . .	·306 „	Total chlorides, . .	·4982 „
Free acidity, . . .	·018 „	Combined chlorides, .	} ·4812 „
Free organic acids, . .	—	Fixed chlorides, . .	

No growth.

Four days later the following amounts were discovered:—

Total acidity, . . .	·216 per cent.	Free HCl, . . .	·048 per cent.
Combined acidity, . .	·168 „	Total chlorides, . .	·3744 „
Free acidity, . . .	·048 „	Combined chlorides, .	} ·3174 „
Free organic acids, . .	—	Fixed chlorides, . .	

One species of organism alone appeared on the plates; it was non-motile, and was 3 μ in length, sometimes more; 1·5 μ in breadth, when stained with methyl-violet. On plates it formed a thick creamy growth, which liquefied the gelatine very slowly. Under the low power the colonies were very similar in appearance to leucin balls, dark brown, circular, and striated with irregular edges.

On agar-agar a slow white dry growth formed of large bacilli made its appearance; the bacilli were 3 to 4 μ long; 1.5 to 1.75 μ thick; ends rounded; chains and heaps; while on potato a yellow-brown dry growth formed, the centre darker than the edges.

17. *A thin leptothrix*.—This form is possibly the same as No. 5, previously described; it, however, seemed smaller and more slender, varying from .1 to .2 μ in breadth. It was grown from the same patient as No. 16, but on still another occasion, when the total acidity was again .216 per cent., the free HCl .0312 per cent., and the organic acids .0048 per cent. Small white ill-defined colonies grew in the gelatine without liquefying it, and, in bouillon, formed small white cottonwool-like masses. Its further properties were not investigated.

18. *A bacillus similar to the B. coli communis, but with much more uneven and irregular margins in the growth on gelatine* (grown from the stomach, neutral and HCl free contents of an alcoholic patient).—The edges of a stroke cultivation on gelatine were excessively contorted, convoluted, and irregular. Milk was rendered acid and coagulated. The bacilli were not motile, or only slightly so. Stained with methyl-violet the length of the bacilli was 2.5 μ , the breadth 1 μ , the ends round. The other properties were not investigated.

19. *Bacillus liquefying gelatine rapidly with a yellow colour*.—This organism was 2 μ long and .9 μ broad, and was non-motile. It quickly liquefied gelatine, changing it into a turbid yellow fluid with a putrefactive odour. On agar-agar, slightly yellow mucus-like growths formed.

20. *Aspergillus niger* (from a case of cancer of the pylorus).—The acidity of the contents of the stomach was .27 per cent., no free HCl, but with lactic and butyric acids present. It liquefied gelatine, giving it an alkaline reaction.

21. *Bacillus* (from the same case but on another occasion, the acidity then being .28 per cent., with no free HCl, and .0652 per cent. of lactic acid).—The organism formed a slightly fluorescent or opalescent growth on gelatine, spreading out but with sharply-defined edges.

Under a high power short bacteria, with rounded ends, almost micrococci, were seen slightly motile. On potato they formed a yellow moist growth, raised above the surface. Milk was slightly coagulated. The cultivation in all media had a cheesy smell.

22. *Bacillus septicus agrigenus?* (from another cancer patient, the amount of acidity present being .244 per cent., with no free HCl).—A delicate growth in stroke cultivations rapidly spread over the gelatine, the edges curved, but barely visible; non-liquefying. On agar-agar they formed round colonies like drops of cream, sticky and tenacious. On bouillon they grew rapidly, forming larger and shorter threads; no spores, non-motile. The bacilli were 3 to 4 μ in length by 1 μ in breadth, occurring singly or in groups.

23. *Large motile bacillus, liquefying gelatine slowly* (from the same case as the last).—The growth of this organism formed a pellicle on the top of the liquefied gelatine. In puncture cultivations this took the form of a white skin, clear in the centre and white about the edges. The bacilli were very actively motile, but were not further investigated.

24. *Bacterium lactis aërogenes* (from the same patient, and at the same time as Nos. 4 and 5).—The growth presented all the familiar characteristics of this organism,—that is to say, the bacilli were, when fresh, 1 to 2 μ long, .7 μ broad, with rounded ends, and were non-motile. They formed a white nail head growth in gelatine, and a yellowish-white growth on potato, with bubbles of gas under it here and there.

A case of special interest is the following:—

The stomach contents of a patient who had suffered from continued and severe

hiccough for some days were siphoned off. He had not taken anything by the mouth for ten days, but had been fed solely by the rectum. A small quantity of slightly opaque acid fluid was procured, which contained no free HCl, nor any of the organic acids. It had no gastric smell, and was only faintly acid. No peptones or glucose present.

The following organisms were separated:—

1. Pink torula.		3. A small-celled yeast.
2. <i>Bacillus coli communis</i> .		4. <i>Proteus vulgaris</i> .

I had now separated a sufficient number of organisms for my purpose, and I proceeded to examine their products on different media. For this purpose the organisms were grown in bouillon, both aerobically and anaerobically, in milk, in glucose solution, and on gelatine. The details of this investigation would be wearisome, and I simply sum them up in Table I., giving first the result of the analysis of the stomach contents from which the organism was grown, and then that of the growth on the various media.

The numbers placed before the names of the organisms indicate the number in the foregoing list.

A glance at Table I. will suffice to show that the lactic acid, which occurs during the first stages of digestion in the stomach, may readily be formed by the numerous micro-organisms present.

No. 8 was cultivated from a stomach contents containing acetic acid, and when grown on bouillon appeared to form this acid both in the presence and absence of oxygen.

Several of the growths in milk became surprisingly acid—as, for instance, Nos. 5, 21, and 24, the total acidity of the milk being .162, .18, and .162 respectively. When grown on sterilised milk also a number of them produced peptones from the proteids—as, for instance, Nos. 9, 11, 12, 14, 15, and 21. As the other media had peptones added beforehand, no observations of the peptonising power of the germs could be made on them.

The table of acidities found in the various specimens of stomach contents is also instructive, one organism surviving although the total acidity reached .432 per cent., .144 per cent. of which was free hydrochloric acid. Again, the other living organisms were found unaffected by acidities of .288 and .324 per cent., most of the acids in these cases being, however, combined to proteids.

THE EFFECT OF PEPTIC DIGESTION ON MICRO-ORGANISMS.

I now wished to ascertain the behaviour of these organisms under gastric digestion. At present the tendency is to give to the stomach the credit of a greater antisepticity and a less digestive power. Some, indeed, go so far as to assert that it is purely ornamental, a survival from those early times when such an organ was indispensable to the form our ancestors had then attained to. They say it is by no means

TABLE I.—SHOWING THE PRODUCTS OF THE MICRO-ORGANISMS.

Name of Micro-organism.	Stomach Contents.			Products in Bouillon.		In Milk.	In Glucose Solution.	On Gelatine.
	Total Acidity.	Free Acidity.	Organic Acids, etc.	Ærobi-cally.	Anæ-robically.			
1. <i>Bacillus coli communis</i> .	·288	?	No organic acids.	Acid ·108. Lactic acid.	Acid ·126. Lactic and acetic?	Lactic acid.	..	Bubbles of gas.
2. <i>Mucor</i> .	·288	?	..	None.	None.	Slightly acid.	..	..
3. <i>Sarcini ventriculi</i> .	·108	?	..	Becomes acid.	None.	None.	..	Becomes acid.
4. <i>Yeast</i> .	·18	?	Lactic acid. No alcohol.	Neutral.	None.	Trace of alcohol.	Co ₂ and C ₂ H ₆ O.	..
5. <i>Slenderleptothrix</i> .	+ ·18	?	Lactic acid.	Acid ·108. Lactic and acetic acid. Alcohol.	Lactic and acetic acids.	Acid ·162. Acetic?	..	..
6. <i>Proteus vulgaris</i> .	Trace.	0	Only slightly acid.	Alkaline.	Acid ·108. Acetic?	..	..	Alkaline.
7. Small-celled yeast.	324	·036	Acetic acid.	..	..	..	Co ₂ and C ₂ H ₆ O.	..
8. <i>Largeleptothrix</i> .	·324	·036	Lactic and acetic acids.	Acid ·09. Acetic acid.	Acid ·109 Lactic and acetic acids.	Acid.	..	..
9. <i>B. subtilis</i> .	·081	·045	Alcohol	..	..	Acid ·018. Lactic acid. Peptones.	..	Alkaline. Alcohol.
10. <i>Micrococcus candicans</i> .	·18	·03	Traces of lactic and butyric acids	Slightly acid. Lactic acid.	Trace of lactic acid.	Acidity slight.	Ærobi-cally alcohol. Anærobi-cally trace of acid.	..
11. <i>B. Luteus liquefaciens</i> .	·18	·03	Traces of lactic and butyric acids.	Slightly acid.	..	Acid ·072. Peptones.	..	Alkaline.
12. <i>Micrococcus</i> .	·018	0	Trace of butyric acid.	Strongly alkaline.	..	Slightly acid. Trace of Peptones.	..	..
14. <i>Pink torula</i> .	·216	0	Trace of butyric acid.	Neutral.	..	Slightly acid. Trace of Peptones.	Lactic acid. Alcohol	..
15. <i>Bacillus</i> .	·432	·144	..	Alkaline.	..	Acid ·072. Peptones.	Slightly acid.	..
18. <i>Bacillus</i> .	0	0	Neutral.	Alkaline.	..	Slightly acid. Lactic.	Slightly acid.	..
19 <i>Bacillus</i>	0	0	Neutral.	Trace of lactic acid.	..	..	..	..
21. <i>Bacillus</i> .	·18	0	Lactic acid.	Acid ·72. Lactic acid.	..	Acid ·18. Lactic acid. Peptones.	..	..
24. <i>B. lactis aërogenes</i> .	·18	?	Lactic acid.	Lactic acid.	Acid ·108. Lactic and butyric.	Acid ·162. Lactic acid.	..	..

useful. These surely must be vegetarians. They disparage its digestive power; they question its anti-fermentative power.

If the stomachs of carnivorous or omnivorous animals are so useless, it is certain that by this time they would have managed to dispense with them. The stomach acts both as a digestive and as an antiseptic organ.

As is stated at the commencement of this paper, a consideration of Macfadyen's work led me to modify his procedure, and I have already described the method adopted. When my apparatus is used, absolute sterilisation is impossible, but for such experiments rigid sterilisation is not necessary. During life no pure culture of one organism can exist in the stomach, although now and then one variety only can be cultivated from its contents, and the growth of one variety influences that of another, often indeed inhibits it as surely as the most powerful antiseptic. The organism which is best suited to the environment flourishes the best. This is applicable both to their growth in the contents of the stomach and to the fact—noted a few lines above—that, with many organisms present, often only one variety will grow in any numbers, or even at all, on the nutrient media employed. There is something, in fact, that offends their sense of the fitness of things; the gelatine is too strong or too weak, too cold or too hot, too alkaline or too acid, or may be it should be fluid, or firmer, or have some glucose in it, or what not. No argument, then, can be used about the organisms present in the stomach, but only about those capable of cultivation from it, until our methods are more perfect.

To become familiar with the process, and to be able to compare results with Macfadyen's, I used for the first two series of experiments the *Staphylococcus pyogenes aureus* and the *Micrococcus prodigiosus*.

Staphylococcus pyogenes aureus.—The staphylococcus was added in bulk, from a growth in bouillon, to a sterilised nutrient fluid containing bouillon, fibrin, cubes of egg, and albumin and water, to which a small quantity of sterilised pepsine had been added. This was placed in a parchment tube and suspended in 3000 c.c. of a solution of hydrochloric acid. In the four experiments the strength of the acid solution was varied. The acid solution also was used in large quantities in order that the supply might be practically inexhaustible during the experiment, as compared, that is, with the contents of the tube.

The acidities used in this series were:—

1. .036 per cent. hydrochloric acid.
2. .2653 ,, ,,
3. .342 ,, ,,
4. .54 ,, ,,

The acidity of the solution inside on each occasion was .036 per cent. before the parchment tube was immersed in the acid solution. No free acid was present.

The beaker which contained the acid and the tube was then placed in an incubator, the temperature of which was kept constantly between 38° and 40° C. by means of a hot-water jacket.

The details of the four experiments are very clearly shown in Table II.

The fluids both inside and outside were analysed, and the organisms tested at the different hours marked on the Table.

The presence or absence of free hydrochloric acid, lactic acid, and in the fourth experiment the amount of free acid, were estimated whenever the total acidity was titrated, and gelatine tube plates were made at the same time.

The acidities outside remained almost constant during the course of the experiments, and need not be further referred to.

The acidity inside became greater than that out, in all four cases; in the first immediately, in the second and third at the first hour, and in the fourth not until the third hour; that is, that the weaker the acid solution was outside, the sooner did the acidity inside rise above it. On the contrary, however, no trace of free hydrochloric acid appeared inside during the first experiment until the fourth hour, in the other three it appeared from three-quarters of an hour to an hour after the commencement.

The staphylococcus survived 24 hours of the first acidity, which never rose above .108 per cent., with only a trace of free hydrochloric acid, but was killed between the second and third hours in the other three, when the acid varied from .306 per cent. to .414 per cent. In the case in which the total acidity was .414 per cent., about .0935 per cent. was free. It is only rarely that the human stomach contains a fluid of so high an acidity, never in a normal state, unless after a heavy proteid meal, and then the acid is almost all combined.

.324 per cent. of combined hydrochloric acid and .0935 per cent. of free acid sufficed to kill the *Staphylococcus pyogenes aureus* in two hours, but it required a much higher percentage of combined hydrochloric acid alone to kill it, and the free acid present was probably the chief agent at work.

By a simple experiment I was able to prove this. I made a solution of hydrochloric acid, combined with the products of the gastric digestion of fibrin, in which there was no free hydrochloric acid, but, notwithstanding, the total acidity of the mixture was .684 per cent. On sterilising and filtering I obtained a clear, bright, heavy, yellow fluid, in which the staphylococcus grew, not luxuriantly, but steadily.

It is to be noted, then, that this organism is markedly resistant to hydrochloric acid, especially in the combined state. Macfadyen found .3 per cent. was the limit, and he regarded this as free acid. He used gelatine plates; and gelatine is a proteid which combines with hydrochloric acid. His .3 per cent., therefore, was made up of combined and possibly some free acid.

I have grown it in ·684 per cent. hydrochloric acid, all combined, and in a constantly increasing acidity up to ·414 per cent., only ·0935 per cent. of which was free.

Micrococcus prodigiosus.—A similar set of experiments was performed with this organism. Unluckily the pepsine used proved to have been insufficiently sterilised, and a large variety of *Bacillus subtilis* grew in the fluid.¹ The point at which the micrococcus died could, however, be identified, although the presence of the other organism may have vitiated the result to some degree.

Table III. gives the results of this series, including three separate experiments.

The acidities outside were:—

1.	·036	per cent.	hydrochloric acid.
2.	·198	„	„
3.	·144	„	„

The acidities inside ran their usual length, the greater rise in No. 1 being due to the greater concentration of the nutrient material used; that in No. 2 was comparatively weak. The presence of free HCl was detected earliest in No. 3, but it will be noted that in each case the micrococcus had died before the advent of the free acid, unlike the preceding organism. The conclusion one must come to, then, is that this organism is very sensitive to the action of HCl, both free and combined. The limit found by Macfadyen was ·05 per cent., the limit here is between ·108 per cent. and ·162 per cent. HCl, all combined, and after only half an hour's digestion.

Bacillus coli communis.—The details of the experiments on this organism are precisely the same as those of the preceding.

The acidities outside were:—

1.	·108	per cent.		3.	·342	per cent.
2.	·054	„		4.	·414	„

To the nutrient media in Nos. 2 and 4 some milk was added, and they were consequently more concentrated than the other two.

The course of the experiment is shown in Table IV.

It will be noticed that free HCl does not appear in the first two until after the normal period of digestion is over, but that it appears sooner in the last two.

The presence of lactic acid could be distinctly demonstrated in this experiment. It appeared, as a rule, in the solution up to the time when the micro-organisms ceased to grow. The explanation of its disappearance later would seem to be that, owing to dialysis of the lactic acid

¹ The organism which grew from the pepsine in this case, a large bacillus, often in chains, was extremely resistant to the action of acids, as it was still living after digestion for 4 hours with the acidity rising to ·36 per cent. Usually, however, it died before the lapse of 24 hours. It was in all probability an aboriginal denizen of the stomach of the pig.

TABLE V.

Time in Hours	0	1/4	1/2	3/4	1	1 1/2	2	2 1/2	3	3 1/2	4	5	24
Growth of Bacterium Lactis Aerogenes .612													
In a parchment Tube with Solutions of HCl outside, In No. 3 and 4 the Solutions were stronger in & out.													
In 1 the solution inside was very weak. Milk in 3 and 4.													
1. Outside. .038% .266 in ———— .252 out ————													
19/3/91													
2. Outside. .054% .216 in ———— .18 out ————													
18/3/91													
3. Outside. .108% .108 in ———— .072 out ————													
10/3/91													
4. Outside. .504% .038 in ———— .018 out ————													
20/3/91													
0													
Presence of Free Hydrochloric Acid in the Solution	1	-	-	-	-	-	-	-	-	-	-	-	+
	2	-	-	-	-	-	-	-	-	-	-	-	+
	3	-	-	-	+	+	+	+	+	+	+	+	+
	4	-	+	+	+	+	+	+	+	+	+	+	+
Presence of Free Lactic Acid in the Solution	1	+	+	+	+	+	+	+	+	+	+	+	?
	2	+	+	+	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+	+	+	+	+
State of the Organisms,	1	+	+	+	+	+	+	+	+	+	+	+	-
	2	+	+	+	+	+	+	+	+	+	+	+	-
	3	+	+	+	+	+	+	+	+	+	+	+	-
	4	+	+	+	+	+	+	+	+	+	+	+	-
Amount of Free Acid driven off by evaporation at 105°C.	1	0	0	.005	.036	.018	0	0	0	0	0	0	.018
	2	.019	.027	.016	.036	.018	.036	.018	.005	.018	.036	.018	.036
	3	?	?	.054	.054	.054	.054	.054	.018	.054	.054	.054	.054
	4	.004	.19	.324	.342	.278	.412	.396	.396	.396	.396	.396	.396

TABLE VI.

Time in Hours	0	1/4	1/2	3/4	1	1 1/2	2	2 1/2	3	3 1/2	4	5	6
1. Growth of Bacillus Leptothrix in a dialyser with HCl. in Solution outside.	.646												
In No. 2 the fluid was more concentrated than in No. 1.	.504												
1. Outside. .054 %	.468												
in -----	.432												
out -----	.396												
2. Outside. .162 %	.38												
in -----	.324												
out -----	.288												
2. Growth of Bacillus Subtilis in a dialyser with HCl. in Solution outside.	.252												
1. Outside. .072 %	.216												
in -----	.18												
out -----	.144												
2. Outside. .162 %	.108												
in -----	.09												
out -----	.072												
	.036												
	0												
Presence of free HCl. in the Solution	1	-	-	-	-	+	+	+	+	+	+	+	+
	2	1	-	-	-	+	+	+	+	+	+	+	+
	2	1	-	-	-	+	+	+	+	+	+	+	+
Presence of Lactic Acid free in the Solution.	1	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+
State of the Organisms + Alive - Dead	1	+	+	+	+	+	+	+	+	+	+	+	+
	1	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+
Amount of free Acid driven off by evaporation at 100° C.	1	0	0.27	0.18	0.18	0.36	0.36	0.16	0.16	0.18	0.18	0.18	0.18
	2	0	0.72	0.36	0	0	0.72	0.54	0.54	0.69	0.69	0.69	0.69
	1	?	0	0.18	0.05	0.18	0.36	0.54	0.54	0.69	0.69	0.69	0.69
	2	0	0.54	0.54	0.72	0.72	0.9	1.12	1.12	0.9	0.9	0.72	0.72

TABLE VII.

[illegible]

already formed, the remainder, being equally distributed both inside and outside, was too small in quantity to give the reactions required to prove its presence.

The life of organisms themselves corresponds closely with the presence or absence of free HCl. In No. 4 they live longer than in No. 3, but in it, although the acidity in No. 4 is higher, free HCl does not make a decided appearance until the second hour. Judging by the acidities in the other three, I have no doubt that, although no organisms grew at the third hour in No. 1, yet, if more plates had been made, some colonies might have been reared.

The experiment, as a whole, then shows that the organism is fairly resistant to HCl, that it can live through a digestive act for, at least, $3\frac{1}{2}$ hours, with acidities of from $\cdot 144$ per cent. to $\cdot 234$ per cent. with no free HCl, but some lactic acid present.

Also, that it is not killed until the end of the two and a half hours, with an acidity rising to $\cdot 522$ per cent., much of it free HCl, or, again, until the end of the first hour with an acidity of $\cdot 234$ per cent., of which free HCl forms a part.

In Table V. the results of similar experiments on the *Bacterium lactis aërogenes* are depicted.

The acidities outside here were:—

1. $\cdot 036$ per cent.		3. $\cdot 108$ per cent.
2. $\cdot 054$ „		4. $\cdot 504$ „

The media inside the tubes were as before.

The duration of life of the organisms again corresponds very closely with the presence or absence of free HCl. In Nos. 3 and 4 they only lived about three-quarters of an hour, when the acidity had reached $\cdot 234$ per cent. and $\cdot 612$ per cent., with free HCl present. They survived in No. 1 until the fourth hour, but in this the acidity never rose above $\cdot 09$ per cent.; while in No. 2, on the other hand, they died after an hour and a half, when the acidity was $\cdot 108$ per cent., with only a trace of free HCl present. Lactic acid was present in all, but in No. 4 its reaction could only be detected for a very short time.

The *Bacillus leptothrix*, the slender leptothrix, or No. 5 of the foregoing list, was next subjected to the action of the acid.

The acidities outside were:—

1. $\cdot 054$ per cent.		2. $\cdot 182$ per cent.
--------------------------	--	--------------------------

The results are depicted in the upper part of Table VI.

Here again the organisms died as soon as free HCl appeared in the contents of the dialyser tube. Lactic acid was distinctly present at the beginning of each experiment.

The exact acidity at which the organism was killed was as follows:—In No. 1, between three-quarters of an hour and one hour after the commencement, when the total acidity reached $\cdot 144$ per cent., with

only a trace of free HCl present; in No. 2, after an hour and a half, when the total acidity was $\cdot 342$ per cent. again with only a trace of free HCl.

In the same Table the results of the growth of another organism, No. 9 in the list, is depicted. This organism is probably the variety of *Bacillus subtilis* described by Buchner. The cultivation used, one on bouillon, had a very evil smell.

The acidities outside were:—

1. $\cdot 072$ per cent.		2. $\cdot 162$ per cent.
--------------------------	--	--------------------------

Comparison of the results, as shown on this Table, shows that this organism, like the last, died shortly after free HCl appeared in the solution. The contents of the parchment tube continued to smell until, in No. 2, the second hour, or an hour after it was possible to cultivate the organism; in No. 1, for two hours and a half, also about an hour after the probable death of the bacillus.

This bacillus lived, then, in No. 1, for $1\frac{1}{2}$ hours, when the acidity reached $\cdot 216$ per cent., $\cdot 03$ per cent. of which was free; in No. 2, until the end of the first hour, with the acidity $\cdot 342$ per cent., $\cdot 01$ per cent. of which was free.

The presence of organic acids, lactic and butyric, again corresponded with the vitality of the organisms, as may be readily seen by a glance at the Table.

Identical experiments on the *Proteus vulgaris* are summed up in Table VII.; the acidities outside being:—

1. $\cdot 072$ per cent.		3. $\cdot 234$ per cent.
2. $\cdot 108$ „		4. $\cdot 52$ „

This organism was not so resistant to the action of acids as some of the others, the hours at which it died being as follows:—

1. $\frac{1}{4}$ hour, $\cdot 072$ per cent; no free HCl.
2. $1\frac{1}{2}$ hours, $\cdot 126$ per cent. ; trace of free HCl.
3. $2\frac{1}{2}$ „ $\cdot 306$ „ „ „
4. $\frac{1}{4}$ hour, $\cdot 378$ per cent. ; no free HCl.

There was never more than a very problematical trace of lactic acid present in any of this series.

I was unable, from want of time and opportunity, to overtake any more observations on the resistance of the remaining organisms to acids and digestion. Those experiments which I have performed, however, are sufficient, both in number and in similarity, to prove that the anti-septicity of the contents of the normal stomach is less than was thought to be the case from arguments based on experiments with pure HCl. They show also that this discrepancy may be easily explained by the diminished power which proteid hydrochlorides have of inhibiting the growth of organisms.

Progressive diminution in the number of organisms during the digestive act.—Wishing to calculate roughly the gross number of organisms present in the stomach during the successive stages of one digestive act, I performed the following experiments coincident with another investigation. A patient who could remove the contents of his stomach at will, and without discomfort, was given a meal of white fish and bread. Cultivations were made from samples of the contents from the first half hour after the meal, at regular intervals, until the fourth hour. The total acidity rose progressively from almost zero to ·381 per cent. at the third hour, ·216 per cent. of which was combined; falling by the fourth to ·342 per cent., with only ·09 per cent. of combined acidity. The number of colonies, on the gelatine plates, was practically innumerable at the half hour, and remained so until after the first hour, when they rapidly diminished, until, by the fourth hour, only one or two growths were observed.

The effect which the presence of numerous micro-organisms has on digestion.—I was led to perform one or two experiments with regard to this matter by the obvious derangement of the processes of nutrition, which may always be observed in cases of dilated stomach with much fermentation.

Bearing in mind the assertions of Ch. Richet and his school, to the effect that, the *acide chlorhydrique combiné organique* might be a hydrochlorate of leucin, I examined the contents of many stomachs for this substance. I failed to find it.

Evaporation of the stomach contents to a syrup, either without the addition of acetate of lead, sulphuretted hydrogen, and subsequent filtration, or after that process had been gone through, gave no positive result.

In no case of artificial gastric digestion could this substance be identified. Tyrosin was also invariably absent.

The following experiment also had a negative result:—To an ordinary dialyser digestive experiment were added many test-tubes full of different organisms grown from stomach contents. These cultivations included those of the *Bacillus coli communis*, the *Proteus vulgaris*, the *Bacillus subtilis*, etc. The nutrient medium was fibrin, gelatine, and some bouillon, to which a pinch of pepsine had been added. Outside there were 3000 c.c. of a ·3 per cent. solution of HCl. The contents beforehand had a very disagreeable smell.

After 5 hours' digestion, at 40° C., this smell was gone, a gastric smell remaining. On examination, no leucin or tyrosin was found, but considerable quantities of combined hydrochloric acid.

A similar experiment, the details of which I withhold, was performed with the pancreatic ferment and a solution of carbonate of soda. Comparisons were made possible by means of a sterilised control,—that is to say, in the one everything was sterilised beforehand, in the other large numbers of organisms were added.

Micro-organisms, when present in large quantity, decreased the amount of albumin digested to an appreciable degree, while they *appeared to inhibit the formation of leucin and tyrosin.*

A repetition of this experiment gave similar results.

CONCLUSIONS.

The following conclusions may be arrived at from a consideration of the foregoing facts:—

1. Many organisms can grow in the human stomach.
2. Many also can be grown from the contents of the stomach, even when they are very acid.
3. From many cases in which organic acids are present in the stomach contents, micro-organisms can be grown, which produce the same acid on suitable media.
4. Most of the organisms so grown are very resistant to the action of acids.
5. Hydrochloric acid combined with proteids—proteid-hydrochlorides—has little inhibitory power on organisms, in comparison with free hydrochloric acid.
6. More micro-organisms can be grown from the gastric contents, if plates be used, the medium of which does not liquefy at the body temperature, than if plates of gelatine be used. Agar-agar is a good vehicle. Yeasts and mucors, however, are best grown on bouillon, being afterwards isolated on gelatine plates.
7. Micro-organisms, if present in quantity, exercise a deleterious effect on both gastric and pancreatic digestion.
8. Though the fatty acids have a strong antiseptic action, and exert rather a helpful influence over pancreatic digestion, yet the combination of hydrochloric acid with proteids is impeded by them during peptic digestion.
9. Pathogenic organisms, which are usually very markedly affected by hydrochloric acid, may pass through the stomach unharmed, if the meal with which they are ingested be large and chiefly proteid in character. Provided also, it may be from some fortuitous circumstance, that the supply of hydrochloric acid be somewhat deficient. Under these circumstances most, if not all, the secreted acid combines with the contained proteids. It follows therefore, from conclusion No. 5, that organisms of all varieties are more likely to reach the intestines unharmed. Perhaps the most favourable occasion on which this might happen would be at a public dinner, when the gastric mucous membrane has been also partially paralysed by the influence of alcohol.
10. Many non-pathogenic germs do, as a matter of course, pass through the stomach unharmed, or only temporarily inhibited. This accords with Macfadyen's observation, noted above, that the bacteria

of the small intestine are constantly changing in numbers and varieties.

11. In the foregoing experiments the utility of a dialyser in such experiments is demonstrated. It allows the hydrochloric acid to be added slowly and constantly, the speed of the dialysis varying directly, as is probably the case in nature, with the proportion of proteid material inside, and with the strength of the acid solution used outside the tube. It also simulates nature more closely, in that all the hydrochloric acid, which first passes through, immediately combines with proteids, the free acid not appearing inside till later.

12. As regards the life of the micro-organisms, this method of adding the hydrochloric acid almost exactly simulates nature, whatever be the difference in the manner of its secretion. The constant motion of the stomach walls and contents is not obtained, therefore the acidity, especially the combined moiety, rises more slowly. This could be easily remedied by some peristaltic apparatus, similar to that employed by Sheridan Lea.

13. Combined acid, again, although not so powerful as the free against bacteria, is still not without influence. A small quantity of the free acid, if combined acid be also present, is sufficient to kill or inhibit organisms which could resist a similar quantity of the free acid alone.

14. Although bacteria are of no aid to peptic digestion, and a hindrance to the pancreatic ferment if in quantity in the duodenum, they still are of great use in the small intestine, where they control putrefaction. This seems paradoxical, micro-organisms obstructing micro-organisms but assisting digestion. It seems, however, to be true ⁽²³⁾. The organisms which most easily pass the searching examination of the stomach are those which give rise by their growth to the fatty acids, as they are the most resistant to the action of acids. Their products in the small intestine are sufficient to keep the contents of that viscus acid, and they thereby prevent or control putrefaction. In the large intestine the secretion is so alkaline that the putrefactive organisms reassert themselves.

15. Increased putrefaction in the intestinal canal may therefore be due, in some cases, either to insufficient mortality among the putrefactive organisms in the stomach, or to too great mortality among the acid-forming bacteria and yeasts.

16. The lactic acid which appears during the first stages of digestion is due to the action of organisms.

17. The lactic, acetic, butyric, and succinic acids found in gastro-ectasis are due also to organisms which luxuriate in the too stationary contents. The marsh gas, the *Brennender-gas* of the Germans, is probably due to the same cause; in the only case of this character, with which I have had the good fortune to meet, no material for examination could be obtained.

18. With regard to particular organisms, the results obtained

have been already tabulated on p. 290, and need not again be referred to.

19. Many of them, however, were capable of producing acids anaerobically, from appropriate media.

20. The formation of leucin and tyrosin does not seem to be aided by the presence of bacteria.

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