Research into the chemical processes in the small intestine of man / by Allan MacFadyen, M. Nencki and N. Sieber.

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RESEARCH INTO THE CHEMICAL PROCESSES IN THE SMALL INTESTINE OF MAN. By ALLAN MACFADYEN, M.D. (Edin.), M. NENCKI, M.D., and N. SIEBER, M.D. (PLATE X.)[1].

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WE possess a considerable number of analyses of the bile, and of the pancreatic and intestinal secretions. Of the latter there are, besides the older analyses of Frerichs, Zander, and others, several of more recent date since Villa improved Thiry's method of establishing an intestinal fistula. There exist, also, numerous experiments made *in vitro* as to the action of these secretions upon the food. But since the early and, for that time, excellent research of Tiedemann and Gmelin, only a few and incomplete observations have been made with regard to the processes as they occur *in vivo*. These observations were made mostly upon animals; a few upon human beings with intestinal fistula.

Through the courtesy of Professor Kocher, it was our good fortune to be able to investigate, during a considerable period, the chemical processes in the small intestine of Man. In the surgical clinic of the University of Berne a woman was this year (1890) operated upon for strangulated hernia. On account of gangrene of the strangulated portion of intestine, and acute inflammation of the surrounding tissues, the gangrenous portion had to be removed, and an anus præternaturalis established. The strangulated and excised piece was exactly that portion of the ileum which opens into the cæcum. As a result the food-mass, after it had been subjected to the action of the mucosa of the whole small intestine, flowed out of the mouth of the fistula instead of passing into the large intestine. We had, therefore, the opportunity of studying, as far as we know for the first time in Man, the chemical processes in the *whole* of the small intestine. In the cases of fistula in the small intestine already recorded, it

[1] Professor Nencki desires to acknowledge in this place the financial support afforded him by a grant from the "Elizabeth Thompson Science Fund" in Boston, and to express his thanks to the Board of Trustees of this admirable Foundation.

was always uncertain in what portion of the same the opening was, and to what degree and extent the food-mass might have been further altered in the portion of intestine below the fistula, An exact knowledge of the processes of digestion can be best obtained from the living subject, and it is of great importance to investigate separately the changes undergone by the food in the individual and anatomically different sections of the digestive tract. The knowledge thereby gained would be invaluable in the treatment of intestinal affections. It was considerations such as these that prompted us to undertake the following investigation. We avail ourselves of this opportunity to thank Professor Kocher for directing our attention to the case, and to express our indebtedness to his assistant, Dr Lanz, for his kind help and the attention he paid to our wishes with regard to the patient.

The patient, Magdalene Spycher, was a peasant woman, from Könitz, near Berne, sixty-two years old, thin, and of medium height. Her weight on admission was only 40 kilogrammes. She was received into the surgical wards on the 16th May 1890, and was operated upon on the same day. The subcutaneous cellular tissue was already partly necrotic; the hernia sac, with the portion of omentum contained therein, completely necrotic. The protruding coil of intestine was gangrenous. There was an oblong perforation in the intestine, and it was evident that the protruding coils were cæcum and ileum. On account of the intense inflammation, it would have been dangerous to stitch the large and small intestine together. Accordingly the gangrenous portions were removed, and an anus præternaturalis established. The portion of small intestine excised was about 10 centimetres long, the portion of cæcum about 3 centimetres long. The wound healed rapidly; and on the following day the patient felt better. There were no symptoms of peritonitis, and the temperature remained normal. A quantity of pulpy excrement was discharged from the fistula in the ileum. Three days later (the 19th) a hard stool passed per anum. From the ileum the discharge was diarrhœic; and tinct. opii was administered. The patient thereafter had a good appetite, and felt well. On the 4th of June, the discharge from the ileum, being again diarrhœic, decoctum rhatanhiæ was administered, and the diarrhœa ceased. On the 5th of June the patient was put upon a diet chosen by herself. It was weighed daily, and consisted of the following items:—

Bread, . . .	260 grms.	Peptone (Kemmerich),	20 grms.
Meat, . . .	100 „	Sugar, . . .	60 „
with two eggs.		Milk, . . .	100 „
Barley gruel, . .	200 „	Bouillon, . .	1050 „

This diet was given during the day as follows:—

Morning, 7 o'clock.—350 grms. coffee infusion, 50 grms. milk, one milk-roll, and 10 grms. sugar.

Morning, 10 o'clock.—350 grms. bouillon, one egg, and half a milk-roll.

Noon, 12 o'clock.—360 grms. bouillon, 10 grms. peptone, 100 grms. minced meat, 200 grms. barley gruel, half a milk-roll, and 10 grms. sugar.

Afternoon, 3 o'clock.—350 grms. tea infusion, 50 grms. milk, 10 grms. sugar, and half a milk-roll.

Evening, 6 o'clock.—350 grms. bouillon, 10 grms. peptone, one egg, and half a milk-roll.

As beverage during the day the patient received 200 grms. wine, 200 grms. water, and 20 grms. sugar; during the night, 150 grms. "grog," with 10 grms. sugar. (The "grog" consisted of rum, water, and sugar.)

A short indiarubber tube was inserted into the opening of the fistula. This tube was cleaned and washed out with water daily. The contents of the intestine were collected in a flask, and handed over to us for investigation. The urine passed in 24 hours was investigated daily, and the urea estimated by Hüfner's method. During June and July the contents of the small intestine were collected as discharged, and investigated by us in different directions. We will first describe their external appearance, and then proceed to the examination of the individual constituents.

The amount of matter passing out of the ileum into the cæcum depends upon the consistence of the food-mass. With the above diet, in which nitrogenous food predominated, the intestinal contents were of thin consistence, and contained on an average 5 per cent. of solids and 95 per cent. water. At times they had the appearance of diarrhœic stools, and constipating drugs were administered. When the patient received a vegetable diet the discharge became more consistent, and contained on an average 10 per cent. of solids. Repeated experiments confirmed this observation. After carefully collecting as much as possible of the discharge from the fistula, we found that the maximum amount of the thin discharge in 24 hours was 550 grms, with 4·9 per cent. of solids. The maximum amount of the more consistent and porridge-like discharge was 232 grms., with 11·23 per cent. of solids. The passage of the food-mass into the large intestine is a constant one. During the night it sinks to a minimum, no doubt because the patient broke her fast five times during the day, and at night received only a stimulant. The evacuation took place without the patient being conscious of the act. Two series of control experiments were made in order to determine when the undigested portion of the food passes into the large intestine, and the length of time the food-mass remains in the small intestine. In the first case the patient was given boiled, unmashed green peas, as we noticed that they were discharged from the fistula unchanged; the second test used was salol, which is decomposed in the intestine, and salicylic acid set free. On the 10th July the patient received at midday (12 o'clock), instead of the barley gruel, 200 grms. boiled green peas. The first discharge

of peas from the fistula took place at 5.30 P.M., the last at 11 A.M. on the next day. They were undigested. The pea diet had, however, to be suspended, as the patient complained of loss of appetite.

On the 28th July she was given, at 10.30 A.M., 2 grms. Salol. Up to 12.15 the intestinal contents were collected as discharged, in all 30 grms. They were filtered, the filtrate acidified with a few drops of hydrochloric acid, and extracted with ether. The ether was then evaporated, and to the remainder were added a few drops of water and a drop of ferric chloride. The result was negative; no salicylic acid was present. The intestinal contents collected between 12.15 and 1.15 P.M. (62 grms.) were treated in the same manner. With ferric chloride they gave a distinct violet colour. The discharge between 1.15 and 3.15 P.M. (33 grms.) contained the largest amount of salicylic acid. We obtained from the ethereal extract a few milligrammes of crystalline salicylic acid. The acidity of the discharge was normal; estimated as acetic acid it was 0·0924 per cent. From this stage onwards the amount of salicylic acid diminished. The last distinct reaction was obtained from the intestinal contents discharged between 12.30 A.M. and 2.30 A.M. The discharge between 2.30 A.M. and 4.30 A.M. (17 grms.) gave no reaction. These experiments were repeated at a much later date, viz., on the 15th and 16th October. At the same time we endeavoured to estimate the amount of the hourly discharge into the cæcum. For this purpose the intestinal contents were collected every two hours in weighed glass flasks.

On the 16th October the patient received at 7 A.M. coffee, a milk-roll, and 125 grammes boiled green peas, and at 9 A.M. 2 grammes of Salol. The following table gives the weight of the discharge, as collected every two hours :—

	7–9	9–11	11–1	1–3	3–5	5–7	7–9	9–11	11–1	1–3	3–5	5–7	
15th October,	117 gr.	49	9	49	118	27	64	27	18	10	12	15	515 grms. in 24 hours.
16th October,	0	62	42	55	56	89	40	14	7	21	2	2	390 grms. in 24 hours.

On the above dates the intestinal contents were of thin consistence. The first discharge of peas came at 9.15 A.M., *i.e.*, after an interval of $2\frac{1}{4}$ hours. There was a fresh discharge at 3 P.M. and again between 7 and 9 P.M. The discharge then ceased. After the administration of Salol (9 A.M.), salicylic acid was detected in the intestinal contents discharged between 11 A.M. and 1 P.M. The last traces were found in the portions collected between 5 and 7 P.M. The experiments with Salol tend to prove that the food-mass reaches the large intestine at the earliest three hours after a meal. The experiments with green peas did not give such consistent results. In the one case the peas first appeared after an interval of $5\frac{1}{4}$ hours, in the other after $2\frac{1}{4}$ hours. In the first experiments with Salol the evacuation of the same lasted about 14 hours, in the second only about 9 hours.

The last peas were discharged after 23 hours and 14 hours respectively. These variations depend upon the varying *consistence* of the food-mass, as determined by the amount of absorption taking place in the intestine The shorter the period that the food remains in the intestine the larger is the amount of water it contains. The discharge we examined in July was thick and porridge-like, and contained 9·3 per cent. solids. The discharge on the 15th October was semifluid, and the amount of solids only 4·8 per cent.

Whilst the patient continued to receive the above-mentioned diet, in which proteids predominated, the discharge from the fistula was yellow or yellowish-brown in colour, due to bilirubin. It was generally almost odourless, and had a slightly burning taste. The faint odour it possessed reminded one of volatile fatty acids. More rarely a feebly putrefactive odour was detected, as of indol. The discharge was usually thin and semifluid—at times thick and porridge-like. In the latter case the average amount of solids was 10 per cent.

Plate X. fig. 1, reproduces the microscopical appearance of the intestinal contents during proteid diet; one can easily recognise numerous striped muscular fibres tinged yellow by the bile pigments, detritus, pigment granules, and amorphous albumen. Also mucin, bile-acid flocculi, vegetable fibres, and numerous bacteria.

Fig. 2 depicts the intestinal contents during a diet in which carbohydrates (mashed peas) predominated. The preparation is tinged with iodine. Starch granules prevail, and are mostly stained red, indicating their transformation into amylodextrin. There are also numerous bacteria.

The numerous experiments we have made upon our patient, and also upon animals, prove that bacteria are constantly present in the fresh intestinal contents.

The normal reaction of the food-mass passing into the cæcum was acid. We tested the discharge from the fistula during five months, from the middle of May to the middle of October. During June and July the reaction was tested daily, and only on two occasions was it neutral, viz., after a diet of mashed peas. The filtered intestinal contents were also titrated with normal alkali solution. Litmus or cyanin was used as indicator. The average degree of acidity, calculated as acetic acid, was 1 per 1000. We will return to this point later on.

The intestinal contents, after filtration from the morphotic and undissolved constituents, contained the following substances in solution:—albumen, coagulable by heat; mucin, peptones, starch-derivatives, such as dextrin and dextrose; ordinary lactic acid and the active paralactic acid; small quantities of volatile fatty acids, chiefly acetic; bile acids

and bilirubin. The discharge became green when exposed to the air. This is due to the transformation of bilirubin into biliverdin.

When the degree of acidity of the filtrate was considerably higher than 1 per 1000—1·5 per 1000—the addition of acetic acid caused no precipitate, or at most a faint cloudiness. By lower degrees of acidity, a flocculent precipitate of mucin ensued. On account of the acidity, the albumen in the filtrate coagulates simply on heating. By higher degrees of acidity the filtrate had to be neutralised with an alkali before the albumen could be precipitated by heat.

The following extracts from our laboratory notebook will serve to give an approximate representation of the percentage amount of albumen, sugar, and acid in the intestinal contents:—

Discharge on 16th June.—Peasoup-consistence, in filtrate albumen, mucin, and sugar. The albumen coagulates on heating. Acidity, calculated as acetic acid, 0·116 per cent.

24th June.—Amount received from hospital = 290 grammes. Pea-soup consistence. Reaction, acid. Acidity = 0·191 per cent. Sugar (Fehling's method) = 1·47 per cent.

25th June.—Discharge semifluid. Reaction, acid. Acidity = 0·171 per cent. Sugar = 0·31 per cent. Coagulable albumen = 0·698 per cent.

29th June.—Discharge semifluid, odour of volatile fatty acids. Reaction, strongly acid.

Microscopically: Numerous muscular fibres, visible also micro-scopically as bile-tinged clots; numerous bacteria non-motile, probably from the high acidity of the discharge.

Sugar = 4·75 per cent. Acidity = 0·207 per cent. The filtrate, on adding acetic acid, gave no mucin precipitate. On heating, the albumen first coagulated after adding ammonia.

30th June.—Discharge more consistent—an odour of fatty acids. Acidity = 0·091 per cent.

1st July.—Amount received = 316 grammes. Odour of fatty acids; semifluid. Acidity = 0·114 per cent.

July 2.—Acidity = 0·154 per cent.; Albumen = 0·45 per cent.

July 3.—Amount = 323 grammes; acidity = 0·122 per cent.; albumen = 0·814 per cent.; sugar = 1·53 per cent.

July 4.—Amount = 228 grammes; peasoup consistence; an odour of fatty acids; acidity = 0·041 per cent.; sugar = 1·29 per cent.

A portion was reserved for the estimation of nitrogen, oxygen, and dry remainder—2·3586 grammes were dried at 110° C. to a constant weight in a small platinum vessel; remainder = 0·1935 grm. and solid remainder = 8·2 per cent. 0·1935 grm. of the solid remainder yielded 9·8 c.c. N. gas at 22° C. and 713 mm. barometric pressure. This is equivalent to 5·39 per cent. nitrogen in the dry solid remainder, or 0·44 per cent. nitrogen in the fresh intestinal contents.

July 7.—Discharge semifluid; faintly acid and almost odourless; a marked peptone reaction; sugar = 1·58 per cent.

2·9276 grms. dried at 110° C. yielded 0·1937 grm. = 6·52 per cent. dry substance. After combustion the amount of nitrogen gas was 12·4 c.c. at 21° C. and 707 mm. barometric pressure. The percentage amount of nitrogen in the dry remainder was therefore 6·78 per cent.; in the fresh intestinal contents 0·44 per cent.

From the 10th to the 18th July the patient was given at noon boiled peas instead of barley gruel. On the first day she complained of loss of appetite, so the peas were given to her mashed—140 grammes daily.

Discharge on July 10.—Two portions were received from the hospital: (1) 120 grammes semifluid discharge, which was passed after the usual diet of meat and barley gruel. The reaction was acid. Sugar = 1·8 per cent. (2) 83 grammes thick consistent discharge, passed at 5.30 P.M., after the patient partook of boiled green peas at 12 o'clock noon. It was filled with swollen undigested peas. The reaction was acid.

July 11.—Discharge porridge-like. Acid reaction. Microscopically were seen numerous cells filled with starch granules, more rarely muscular fibres. A portion of the discharge was semifluid. The whole was mixed with water and filtered. In the filtrate were sugar and albumen. On adding acetic acid, no cloudy precipitate.

July 12.—The discharge was thinner and more fluid. Numerous cells containing starch were present. Acidity = 0·163 per cent.

July 13.—Reaction acid; odour, faintly putrefactive. In the filtrate was 0·95 per cent. sugar.

July 14.—Discharge thickish; acid, with a faint putrefactive odour. A portion was dried for an elementary analysis.

3·3624 grammes dried in a platinum vessel gave 0·2982 grm. remainder. Dry remainder therefore = 8·87 per cent. The nitrogen estimation gave 12·6 c.c. N gas at 22° C., and 713 mm. bar. pressure, *i.e.*, 4·49 per cent. N in the dry remainder, or 0·398 per cent. N in the fresh moist substance.

Further, 3·4518 grms. moist substance gave 0·3062 grm. dry remainder. The combustion in an oxygen current with CuO, gave 0·4987 grammes CO_2, and 0·167 grm. H_2O = 44·41 per cent. C. and 6·05 per cent. H.

The remainder of the discharge, as well as that of the 14th, 15th, and 16th July, was dried and utilised for an ash estimation.

July 18.—Discharge thickish, reaction acid. It contained numerous muscular fibres, and was the first passed after stopping the pea diet.

July 19.—Discharge thickish and consistent, and contained many muscular fibres. The filtrate was acid, and contained only traces of sugar and albumen. The albumen was first precipitated after adding ammonia. Acetic acid gave no cloudy precipitate.

July 23.—Discharge consistent and acid—sugar = 0·47 per cent. The solid remainder was estimated, as well as the substances soluble in ether. 2·453 grammes gave 0·2754 grm. dry remainder = 11·23 per

cent. Further, 5·1505 grammes gave 0·5785 grms. dry remainder and contained 0·047 grm., *i.e.*, 8·12 per cent. of matter soluble in ether.

July 27.—Amount of discharge received = 385 grms. It was semi-fluid, and contained 0·937 per cent. sugar. Acidity = 0·154 per cent.

July 30.—Amount was 307 grms. with 9·12 per cent. solid remainder.

July 31.—Amount = 269 grm. with 9·29 per cent. solid remainder.

The discharge on the last two days was used for an ash estimation.

The figures we obtained show that the amount of dissolved albumen which passes with the food-mass into the cæcum is less than 1 per cent. The amount of sugar is subject to greater variations,—from 0·3 per cent. to 4·75 per cent. The maximum amount of sugar in the discharge was found on the 29th of June, and on that day the acidity also reached its maximum, viz., 0·21 per cent. The intestinal contents were watery and diarrhœic in character. We always found that the amount of sugar and acid was higher in the semifluid than in the more consistent and porridge-like discharge. In the latter case the absorption was clearly greater.

After a diet of meat, eggs, peptone, and barley gruel, the amount of unabsorbed nitrogen, *i.e.*, albumen, was equal to 5·39 and 6·78 per cent. of the dry remainder. When mashed peas were substituted for barley gruel, the most of the starchy matter passed unchanged through the small intestine, and the amount of nitrogen was 4·49 per cent. This nitrogen was almost entirely derived from albumen.

On adding caustic soda to the intestinal contents no smell of ammonia was detected. On heating there was a faint odour of ammonia and trimethylamine. As will be shown further on we found neither leucin nor tyrosin in the discharge. If we calculate the nitrogen as albumen, by multiplying with the factor 6·25, then 5·39 grms. nitrogen = 32·68 grms. albumen; 6·78 grms. N = 42·37 grms. albumen and 4·49 grms. N = 28·0 grms. albumen. According to this calculation, 30–42 per cent. of the dry remainder consisted of proteids. If we add thereto 8·5 per cent. for inorganic salts, and an equal amount for fat and substances soluble in ether, about 40 per cent. of the dry remainder would consist of carbohydrates and substances only soluble in alcohol. With a diet of mashed peas the amount of carbohydrates would be about 55 per cent.

One fact is especially worthy of notice, viz., our observation that the contents of the small intestine in its entire length have an *acid* reaction. In animals, and in cases of fistula in the small intestine of man, this observation has repeatedly been made. Tiedemann and Gmelin[1] state that in fasting animals the fluid contained in the small intestine reddened litmus paper, and that this acid reaction diminished towards the lower part of the intestine. The same authors confirm the fact, first observed by Prevost and Le Royer,[2] that the food-mass in the first two stomachs of ruminants has an *alkaline* reaction. In the third stomach the contents were thinner and reddened litmus paper, similarly also in the rennet stomach, and in the whole of the small intestine to the end of the ileum, where the acidity completely disappeared. In the Carnivora, according to Meissner, the contents of the duodenum have always an acid reaction. Ewald states that in his patient, with a fistula probably in the lower part of the small intestine, the reaction of the fresh discharge immediately tested was neutral or faintly acid, but at no time alkaline. We may mention here that the intestinal contents should first be filtered before testing the reaction or estimating the acidity. This removes the deeply bile-stained constituents of the food-mass, which tend to mask the reaction. It often occurred that whilst the reaction of the fresh discharge seemed to us doubtful, the filtrate had a distinctly acid reaction. The causes of the acid reaction of the contents of the small intestine down to the cæcum, are undoubtedly organic acids, and chief amongst these acetic acid. The lactic acids formed in the digestive tract are neutralised by the alkali supplied by the mucosa. The neutralisation of the hydrochloric acid of the gastric juice takes place in the upper part of the intestine. We repeatedly tested the filtered intestinal contents with the methyl-violet and the Ginsburg reagent for free hydrochloric acid. The results were always negative.

All analyses of the intestinal juice consistently show that this secretion contains sodic carbonate, and it was interesting to note in our patient that the mucous coat of the ileum reacted alkaline, the food-mass covering it acid. Incidentally we observed

[1] Berzelius' *Jahresbericht*, vol. vii., 1828.

[2] Berzelius' *Jahresbericht*, vol. v.. 1826.

that the alkaline reaction of the colon's mucosa was always more intense than that of the ileums. The statement found in most handbooks that the chyme is already neutralised in the upper part of the small intestine, and generally reacts alkaline in the lower part, is not correct. The alkaline reaction first begins in the large intestine, after the food has passed through the ileo-cæcal valve. Through the continuous neutralisation of the chyme on the alkaline mucous coat of the small intestine a precipitate results. This precipitate consists of mucin, bile acids, fat, cholesterin, and a neutralisation precipitate of albumen. This precipitate adheres to the mucous coat, and this circumstance may well be of importance for the absorption of fats.

When we mixed the filtered discharge from the fistula with a 5 per cent. solution of glycocholate of soda, a precipitate did not immediately occur; but after half an hour all the glycocholic acid was precipitated.

The fact that the food-mass in the entire length of the small intestine has an acid reaction, and that, consequently, the pancreatic digestion of proteids, carbohydrates, and fats takes place in an acid medium, should be taken into account in future artificial digestion experiments. It has been already shown by one of us[1] that by *acid* reaction the pancreas splits into their components, fats and acid ethers or "esters." Experiments, to be immediately described, show further, that the acid reaction of the food-mass has a distinctly inimical influence on those bacteria which only flourish in neutral or alkaline nutrient media.

It was of especial interest to discover the share the numerous bacteria present in the small intestine take in the decomposition of the food. The acid reaction of the food-mass and its faintly and not always putrefactive odour, were already against the supposition of any active decomposition by their agency. Still it was possible that, whilst on account of deficient oxygen the final products of putrefaction—such as indol, skatol, phenol, volatile fatty acids, &c.—would not be found, the first decomposition products of the proteids (the amido acids) might be present.[2]

[1] *Archiv für. Exper. Pathol. u. Pharmakol.*, vol. xx. p. 375, 1885.

[2] *Wiener Akad. Berichte*, 1889.

To investigate this point we proceeded as follows:—The intestinal contents daily received from the hospital were at once mixed with oxalic acid; the amount of acid added was 5 per cent. The discharge was collected for several days, and then distilled, about a kilogramme at one time. A small flask connected with the condenser collected any volatile products carried over with the water vapour. The gases developed during the distillation passed from this flask into a glass-bulb apparatus filled with a 3 per cent. solution of the cyanide of mercury. This solution would absorb sulphuretted hydrogen and methylmercaptan if present. In this manner we manipulated more than two kilogrammes of the intestinal contents. As regards gases, with the exception of CO_2, only traces of H_2S were present. The amount was so small that it was only after long standing that any precipitate of sulphide of mercury took place in the solution. The first portions of the watery distillate, tested with picric and hydrochloric acids, or nitrous acid, gave neither an indol or skatol reaction. Bromine gave no precipitate, and only Millon's reagent produced a faint pink tinge in the distillate after heating. Thus the final products of the putrefactive decomposition of albumen failed entirely, or were only present in traces. This agrees with Ewald's results. He found in the intestinal contents of his patient neither phenol, indol, or skatol. That a minimal amount of indol was present was proved by the odour of the discharge, and the presence of indoxyl in the urine. These two tests are much more delicate than picric acid or nitrous acid. We detected indigo in the urine of the patient on different days, after the large intestine had been empty for more than a month. On adding to the urine an equal volume of chlorine containing hydrochloric acid and shaking with some chloroform, the latter acquired a distinctly blue colour, due to the dissolved indigo. The addition of chloride of calcium is to be avoided, as it destroys any traces of indigo which may be present. From two kilogrammes of the intestinal contents we obtained about 1·5 gramme of volatile fatty acids, consisting almost entirely of acetic acid. The soda salt was precipitated with a solution of nitrate of silver, and the silver salt analysed. Result = 64·1 per cent. Ag. The formula $C_2H_3O_2Ag$ corresponds to 64·67 per cent. Ag.

The remainder in the retort, after distilling over the volatile products, was concentrated on the water-bath to a syrupy consistence, and then extracted with ether. After distilling off the ether there remained a small quantity of an acid syrupy fluid. This acid fluid was miscible in all proportions with water, and gave negative reactions for phenylpropionic, skatolacetic, and aromatic oxyacids. It was boiled with an excess of zinc hydroxide, filtered, and the filtrate concentrated on the water-bath till crystallisation began. The first zinc salt which crystallised out of the cooled solution resembled microscopically the lactate of zinc. It was recrystallised out of water, and analysed. 0·2253 gram. of the salt dried in the air lost, at 110° C., 0·0411 grm. in weight = 18·24 per cent.; and 0·1842 grm. of the dry salt after combustion gave 0·0612 grm. ZnO

= 26·66 per cent. zinc. The salt was therefore the ordinary lactate of zinc, with three molecules of water of crystallisation. The formula $(C_3H_5O_3)_2Zn3H_2O$ requires a loss of 18·18 per cent. in weight through water of crystallisation. The mother liquid of these crystals, on being further concentrated, deposited a second zinc salt, easily soluble in water. This led us to suppose that the optically active paralactic acid might also be present. By repeated crystallisation in water we were able also to obtain this easily soluble salt in a pure state. After drying in the air, 0·2008 grm. lost at 110° C. 0·0257 grm. weight = 12·88 per cent.; and 0·1751 grm. of the dry salt gave on combustion 0·0583 grm. ZnO = 26·72 per cent. zinc. The paralactate of zinc contains 12·89 per cent. water of crystallisation. Two kilogrammes of the intestinal contents yielded approximately three grammes of the lactic acids, and each of the two acids was present in about equal amount.

The complete absence of the fermentation products of albumens prompted us to search for the next hydration products of proteids, viz., leucin and tyrosin. On four successive days the fresh intestinal contents were mixed with three times their volume of absolute alcohol, then filtered and the alcohol distilled off. The remainder in the retort, after evaporation, showed no crystalline remainder after long standing. The portion insoluble in alcohol was next extracted with warm water and filtered. Lime and phosphoric acid were removed by adding a small quantity of carbonate of ammonia, and the liquid condensed to syrup consistence. After long standing neither leucin nor tyrosin were detected. The syrupy mass was then extracted with hot alcohol, and the filtrate condensed on the water-bath. Here also, even after two months, no crystallisation occurred. The syrupy mass consisted chiefly of peptones, mixed with sugar and bile acids.

We now followed another method. A larger quantity of the intestinal contents was extracted with ether, in order to remove the bile constituents and fats. 233 grammes of the dry intestinal contents were treated with ether in an extraction apparatus. Over night well-formed rhombic crystals of bilirubin crystallised out of the ethereal solution. The faintly-red solution contained no urobilin, and, examined spectroscopically, gave no absorption bands between *b* and *F*. The addition of an alcoholic-ammoniacal zinc chloride solution did not produce the slightest fluorescence. This observation is interesting, because it shows us that the seat of the reduction processes, especially the metamorphosis of bilirubin into urobilin, is not in the small but in the large intestine. As ether did not completely remove the bile pigment, the powder was once more extracted with chloroform. In this case also only bilirubin was found, and no urobilin. We next extracted with alcohol, and evaporated the alcoholic solution. As it still contained gummy bile substances which gave a precipitate in water, the remainder was boiled with water and the watery extract evaporated. After standing for several days a few rhombic needles formed, which might easily be mistaken for tyrosin. They were freed as much as possible from the mother

liquid. They were easily soluble in water, and gave all the reactions of succinic acid. The weight of the remainder, after complete extraction with ether, chloroform, and alcohol, was 201 grammes. Thus the dry intestinal contents yielded 13·3 per cent. of soluble substances. The watery extract, condensed to a syrup, gave neither leucin nor tyrosin. It consisted chiefly of sugar and peptones. We will not positively assert that leucin and tyrosin are not formed out of proteids in the small intestines by the action of the pancreatic juice. If, however, these two substances do arise in the small intestine, their amount must be very small and their absorption very rapid.

Our investigations show that in the small intestine the proteids are not, or only in very small quantity, decomposed by the bacteria. The faintly acid reaction of the intestinal contents suggested, however, the possibility of a decomposition of the carbohydrates through their agency. The presence of lactic and acetic acid also favoured this assumption. Microscopical preparations of the discharge abundantly proved that microorganisms were present in great number, and our next task was to isolate the microbes in pure cultures, with a view of testing their action on proteids and carbohydrates. The opening of the fistula was always kept aseptic, and the woman was in a private room and separated from the other patients. We were, therefore, able to carry out our bacteriological investigations under favourable conditions, and to make the inoculations in a quiet atmosphere. The patient by coughing was able to move the food-mass downwards, and to eject a portion from the mouth of the fistula. With a sterilised platinum loop it was easy to reach the lumen of the intestine, and to remove portions of the contents before they came in contact with the air. At times the discharge was so abundant that we could collect it directly in a sterile glass vessel, which was applied closely to the mouth of the fistula. The further investigation was carried out as follows.

Coloured and uncoloured preparations of the fresh discharge were examined microscopically, in order to obtain a preliminary notion of the bacterial forms present, and their relative number. Portions of the intestinal contents were well mixed with liquefied gelatine (10 per cent.) and agar (1·5 per cent.). From these Esmarch "roll-plates" were made in 5 to 6 dilutions. The gelatine and a portion of the agar-plates were kept at 18° to

20° C.—the remaining agar-plates were placed in an incubator at 37° to 38° C.

Portions of the discharge were also mixed with a weakly alkaline meat broth, and after one to two days gelatine and agar-plates made therefrom.

Anaerobic plates were also made. The agar and gelatine after inoculation were poured out in small glass vessels, and covered with a layer of gelatine or liquid paraffin. Esmarch "roll-plates" were also covered with sterile paraffin or olive oil. Nutrient solutions, containing glycerine, grape-sugar, and bile respectively, were also used. We will return to these later on. We made inoculations twice whilst the patient received a diet in which proteids preponderated, and once whilst the diet consisted in great part of carbohydrates (mashed peas).

I. *The Cultures after a Meat Diet.*

The coloured and uncoloured microscopical preparations contained a great number of bacteria. Amongst these were isolated forms, which remained uncoloured, or only faintly stained, by the pigments (methylene blue and phenolfuchsin). It was not easy to distinguish the different forms from one another. We could, however, recognise with certainty four forms of bacilli and two species of micrococci—in all six. Examined in meat broth in a glass cell, some of the bacteria were motile. The majority were non-motile, and seemed to us to be in an enfeebled condition. The gelatine plates were next examined. The first dilutions were quickly liquefied, and it was difficult to separate the different colonies from one another. From the more dilute plates we could, however, isolate the different colonies. The liquefying bacteria were present on all the plates, and as they grew more quickly they were first isolated. They were bacilli, and in "stabcultures" produced a funnel-shaped liquefaction of the gelatine. The agar-plates and those made from meat broth rendered it easier to isolate the more slowly-growing forms. We examined the plates so long as there was any apparent growth. By these methods we isolated eight bacterial forms, three of which were distinguished by their constant presence on the plates. These were:—

1. A bacillus, which quickly liquefies gelatine, and which we will call the *bacillus liquefaciens ilei.*
2. A short rod-shaped bacillus, resembling in appearance the bacillus coli commune.
3. An oval bacterium, which did not liquefy gelatine.

In addition to these predominating forms, the following microbes were isolated from scattered colonies :—

4. A bacillus of ellipsoid shape.
5. A large, plump bacillus.
6. A streptococcus, non-liquefying.
7. Yeast fungi.
8. A mould fungus, resembling morphologically, and probably identical with, the oidium lactis.

It is worthy of notice that not only schizomycetes, but also yeast and mould fungi, were isolated from the contents of the small intestine, and that they still retained their vitality after having been subjected to the action of the gastric and intestinal juices. The three first-mentioned and the yeast fungi were the only forms present in number; the others were first isolated after many examinations of the plates. Putrefactive bacteria were not isolated. On the anaerobic gelatine and agar plates we found three forms, which, however, in their morphological appearance and growth in different nutrient media, corresponded to forms already isolated aerobically. They were :—

1. A non-liquefying short rod bacillus.
2. A non-liquefying oval bacterium.
3. A streptococcus.

The first two were the prevailing forms. All three were facultative anaerobic bacteria. Amongst the bacteria isolated we found no obligatory anaerobic forms.

II. *The Cultures after a Diet mainly Carbohydrate.*

The methods were the same as in the former case. The microscopic preparations again contained numerous bacteria. The coloured preparations contained also forms which took up the pigment very feebly or not at all. We could distinguish five different forms with certainty; three were bacilli and two micrococci. Some of the bacteria were motile. Here also the first plates made were quickly liquefied. The liquefying microbe was isolated from the more diluted plates, and proved to be a streptococcus, and not the bacillus isolated during meat diet. There was, secondly, a slender bacillus most constantly found on the plates. It was not observed on the plates made whilst the patient was upon meat diet. Yeast fungi were numerous.

We isolated in pure cultures from the agar and gelatine plates the following micro-organisms :—

1. A bacterium, resembling closely the bacillus coli commune, and probably identical with the bacillus already isolated during meat diet.
2. A non-liquefying diplococcus.
3. A non-liquefying diplococcus, smaller than the former.

Three forms were isolated from the anaerobic plates, viz., two species of cocci, morphologically identical with those already isolated aerobically. The third form was a bacillus, not found on the aerobic plates. It was a long and rod-shaped bacillus, and the bacilli were arranged in circular chains. It was not found on the plates during meat diet. In pure culture it grew also aerobically. We isolated altogether the following forms, amongst which were no putrefactive bacteria :—

1. A liquefying streptococcus.
2. A slender rod-shaped bacillus.
3. A large diplococcus.
4. A small diplococcus.
5. Bacterium, resembling bac. coli commune.
6. A "chain" bacillus.
7. Yeast forms.

Mould fungi were in this case not found.

We noted that with a change of diet, and after a lapse of time, quite different bacteria predominated—in this case, for example, the streptococcus liquefaciens and the slender rod bacillus. Of those isolated during meat diet we found only the bacterium, resembling the bac. coli commune. All the bacteria isolated were facultative anaerobic ; they grew aerobically and anaerobically.

III. *Cultures after a Meat Diet.*

Four weeks later, when the patient was again upon meat diet, inoculations were made for a third time from the intestinal contents, and the usual series of cultures therefrom.

As formerly, there were numerous bacteria present in the food-mass, and it was possible to distinguish six forms. There were also bacteria, which coloured badly or not at all, and motile bacilli. On the plates there was no general liquefaction of the gelatine, so that we were led to suppose that the liquefying forms already isolated were only sparingly represented in the intestinal contents at this time. Two forms were constantly found—a non-liquefying bacillus, with rounded ends, and an oval bacterium. Complementary experiments were now made

with sugar and glycerine gelatine. In all, seven forms were isolated :—

1. A rod bacillus, with rounded ends.
2. An oval bacterium.
3. A short rod bacillus, with flattened ends.
4. A micrococcus, which liquefies gelatine slowly and partially.
5. A short and thick bacillus, with rounded ends.
6. Yeast fungi.
7. A mould fungus.

The first two forms were present on all the plates, and especially numerous on the plates made with sugar and glycerine gelatine. On the anaerobic plates there were colonies of a bacterium, about the same size as the Bact. coli commune. It, however, slowly liquefied the gelatine. Here, again, all the micro-organisms were facultative anaerobic. On the whole the picture was different to that presented in the first and second series of experiments. The bacterial forms present in the small intestine seem to be in a perpetual state of change. After a lapse of time, and after change of diet, different forms prevail, and the previously predominating forms are pushed into the background or completely disappear.

These preliminary experiments being finished, our next object was to study more closely the morphology and physiology of those bacteria which were most constantly present in the intestinal contents, and which consequently might be regarded as typical forms. We hoped in this way to be able to arrive at a definite and just conclusion as to the share the bacteria take in the digestion and decomposition of the food in the small intestine.

The bacteria selected for the above reasons were :—

1. The bacterium resembling, and perhaps identical with, the bact. coli commune. (Meat diet, I.)
2. The streptococcus liquefaciens ilei. (Carbohydrate diet.)
3. The bacterium ilei. (Meat diet, II. 2.)
4. The bacillus liquefaciens ilei. (Meat diet, I.)
5. The oval bacterium or bacterium ovale ilei. (Meat diet, I. 3.)
6. The slender rod bacillus, or *bacillus gracilis ilei.* (Carbohydrate diet.)
7. The short rod bacillus, probably identical with the bacterium lactis aerogenes of Escherich. (Meat diet, II. 5.)

I. *The Bacterium resembling the Bacillus coli commune.*

It was given to Dr Bischler for chemical investigation, and accordingly we named it the "Bacterium Bischleri." It is a short rod-shaped bacillus. It varies greatly in size, on an average it is 4 μ long and 3 μ broad. The bacilli are usually in pairs, and are non-motile. Spores were not observed. In appearance

it resembles closely the bacillus coli communis, and at first we supposed it to be identical with the latter. It does not liquify gelatine. On gelatine the deep colonies have a yellowish colour, darker in the centre, and are finely granulated. The superficial colonies have a dull white colour (*v.* Plate X. fig. 1). The stabcultures in gelatine grow slowly as small dull white granules along the line of inoculation. Superficially the growth is slender, and forms a thin layer covering about two-thirds of the surface of the gelatine. The margin is irregularly curved. In agar the growth is similar. The bacillus coagulates milk at 38° C. within 22 hours. At 15° C. the milk is coagulated after 5 to 6 days. Guinea-pigs, after subcutaneous inoculation, died in 2 to 3 days.

The action of the bacillus on proteids and carbohydrates was next investigated:—

200 grammes dextrose were dissolved in 3 litres of meat broth, and 75 grammes carbonate of lime added. The solution, after sterilisation, was inoculated on the 16th July from a pure culture of the bacillus, and placed in an incubator at 37°to 38° C. Active fermentation set in, and there was an active development of gas. On the 9th day the development of gas abated, and the solution was examined as follows:—First of all its purity was tested, it contained only the bacterium Bischleri. The sugar was next estimated. The fluid reduced very slightly a faintly alkaline solution of copper—and examined in the polariscope there was no rotation. There was therefore only a minimal amount of unchanged sugar present. The fluid was next decanted from the deposit and distilled till the distillate, tested with iodine and caustic soda, gave no further iodoform reaction. On saturating the distillate with calcined potash, an alcohol was obtained, which, after drying over caustic potash, distilled constantly at 77° C. It was therefore pure ethyl alcohol, and about 6 grammes were obtained. The remainder in the retort was treated with oxalic acid, the oxalic acid precipitate filtered off, and the filtrate once more distilled. The volatile products (fatty acids) were exactly neutralised with soda, and the solution condensed on the water-bath. The soda salt was recrystallised out of alcohol, and precipitated with nitrate of silver. 0·2224 grm. of the silver salt from the first crystallisation, left, after combustion, 0·1435 grm. silver = 64·52 per cent. silver. From the second crystallisation 0·2043 grm. of the silver salt yielded 0·1332 grm. silver = 64·7 per cent. The acetate of silver contains 64·6 per cent. silver. The volatile acid was thus acetic acid, of which about 7 grms. was obtained. The remainder in the retort was concentrated to a syrup, and extracted with ether. After distilling off the ether a yellowish syrup remained, which, boiled with zinc hydroxyde, gave ordinary lactic acid. The zinc salt contained 17·98 per cent. of crystallisation and 26·82 per cent. Zn. The theoretical formula requires 18·18 per cent. water, and 26·74 per cent. Zn.

It is interesting to note that Dr Bischler obtained from sugar cultures of the *bac. coli commune* the *same* fermentation products, viz., ethyl-alcohol, acetic and lactic acids. The lactic acid obtained was, however, the *optically active* paralactic acid, with 12·9 per cent. water of crystallisation.

The two bacteria therefore differ from one another, inasmuch as their fermentation products are not wholly identical. The chief point of difference is that in the one case the optically inactive, in the other case the optically active, lactic acid was produced. We proved recently that the so-called sarco- or paralactic acid is formed out of sugar by the micrococcus acidi paralactici.[1] Since then we have found five forms of bacteria which produce from dextrose the optically active paralactic acid. We will describe these forms on another occasion. We may, however, suggest here that the production of the inactive or active lactic acid may prove a useful diagnostic method for distinguishing between individual forms of bacteria. Thus by means of this chemical test we were able to prove that the bac. Bischleri and the bac. coli commune were not identical, though morphologically they resembled one another closely. The bac. Bischleri has no action upon proteids. Finely minced meat was mixed with four times its volume of water and sterilised. After inoculation the culture was made anaerobic by replacing the air with carbonic acid. The flask was kept at 38° C. After seven days no gas developed, and the fluid remained clear. The flask was then opened, resterilised, and inoculated from fresh cultures. It was simply plugged with cotton wool. After 10 days at 38° C. there was no decomposition, the contents of the flask remained clear and odourless. Microscopically the number of bacteria was very small.

II. *Streptococcus liquefaciens ilei and Acidi lactici.*

The micrococci are small and delicate, and the chains often consist of 20 and sometimes 40 members (*v.* Plate X. fig. 2). They were easily stained with the ordinary aniline dyes. In gelatine they formed small round yellowish colonies, surrounded by a narrow zone of liquified gelatine. The "stabcultures" in gelatine were characteristic. On the surface there formed a

[1] *Wiener Akad. Ber.*, 1889.

saucer-like liquefaction of the gelatine. The liquefaction proceeded gradually from above downwards, and after three weeks two-thirds of the gelatine was liquefied. In agar they form a dull greyish-white layer covering the entire surface. In bouillon they grow rapidly. After 24 hours at 38° C. the bouillon is quite cloudy, and after 2 days there is a deposit of bacteria at the bottom of the tube. There was no putrefactive smell. Sterile milk was coagulated after 22 hours at 38° C. Guinea-pigs, inoculated from broth cultures, died after 24 hours.

In order to study the decomposition of sugar and albumen by this and the following microbes, we prepared two kinds of nutrient fluids:—

1. Dextrose, 40 grm.; Kemmerich's peptone, 12 gr.; calcium carbonate, 16 gr.; sodic-chloride, 2 gr.; and water, 800 grm.
2. Finely minced meat, 200 grm; and water, 800 grm.

These solutions were sterilised in flasks plugged with cotton wool. On the 21st August they were inoculated with the respective bacteria, and placed in an incubator at 38° C. On the 12th September they were taken out and kept at 15° C. till October, when they were examined. The sugar solution inoculated with the streptococcus liquefaciens ilei was microscopically examined. It contained only the above micrococci. The further investigation was conducted as described by the bac. Bischleri. There were only traces of unchanged sugar. A small quantity of alcohol was obtained, not sufficient for an accurate estimation of its nature. The concentrated liquid solidified on cooling to a crystalline mass, consisting of the lactate of calcium. A portion of the calcium salt was converted into the zinc compound and analysed. 0·2305 grm. lost at 110° C. 0·0423 grm. in weight, and gave after combustion 0·0623 grm. ZnO., *i.e.*, 18·34 water and 26·57 per cent. ZnO. It was therefore the inactive lactic acid. With the exception of small quantities of bye-products, the sugar was completely transformed into the inactive lactic acid. This microbe appears to be especially adapted to the production of lactic acid in the intestine.

The meat cultures were in part decomposed. The fluid was cloudy, strongly alkaline, and had the odour of old cheese, without reminding one of indol or skatol. Microscopically bacilli were also present. The culture being thus contaminated we did not examine it further.

III. *Bacterium ilei.*

It is a short rod bacillus with rounded ends, 2 to 3 μ long and 1 μ broad. The bacilli are usually in pairs, at times also in groups. They are feebly motile, and form spores usually at the two poles. They are easily coloured with methylene blue and Ziehl's solution. In gelatine the colonies grow well on the

surface, and exceed 5 or 6 times in diameter the deep colonies. They have a greyish-white colour. They are finely granulated, and three zones can be distinguished,—an inner brownish, then a yellowish, and a marginal of a yellowish-white tinge. The margin is irregularly curved (*v.* Plate X. fig. 3). The stab-cultures in gelatine form fine yellowish-white discrete granules along the line of inoculation. Superficially they form a dull white moist and thick layer, with a wavy margin, covering almost the entire surface. In agar the surface growth is similar; the deep growth is yellowish-white, and indistinctly granular. They grow rapidly in bouillon, and at 38° C. coagulate milk within 20 hours.

The sugar cultures on examination were found to be pure, and the fluid odourless. It reduced a weakly alkaline solution of cupric sulphate. On testing with Wild's polaristrobometer, the fluid was lævo rotatary = 40′ in a 100 μ tube.

The distillate gave a marked iodoform reaction. By saturating with potash, and drying and rectifying, we obtained 6 grm. of an ethyl alcohol which boiled between 76° and 77° C. at 706 mm. barometric pressure. The amount of alcohol obtained was equivalent to 15 per cent. of the sugar used. The remainder in the retort was acidified with HCl and extracted with ether, and then with alcohol ether (1 vol. alcohol and 2 vols. ether). We obtained as chief product succinic acid, and in smaller amount the active paralactic acid. After distilling off the ether and adding a small quantity of water, succinic acid crystallised out, whilst the lactic acid remained in solution. The mother liquid was filtered off from the crystals, and the filtrate boiled with zinc hydroxyde. The filtrate contained the soluble zinc lactate, and succinate of zinc remained as an insoluble precipitate on the filter. 0·1893 grm. of the salt lost at 110° C. 0·0239 grm. in weight = 12·62 per cent. H_2O; and 0·1654 grm. on combustion gave 0·0556 grm. ZnO = 27·0 per cent. zinc. The succinic acid was also analysed. 0·2246 grm. of the substance recrystallised out of water gave 0·3368 grm. CO_2 and 0·1083 grm. H_2O, or 40·89 per cent. carbon and 5·35 per cent. hydrogen. The formula $C_4H_6O_4$ corresponds to 40·68 per cent. carbon and 5·08 per cent. hydrogen.

We thought it possible that the second and until now unknown active ethylidene lactic acid might be formed by this bacterium from sugar.

We have accordingly asked Dr Frey to repeat the experiments on a larger scale.

Cultivated anaerobically in sugar, the gases formed were found to be carbonic acid and hydrogen. On the third day the gas collected consisted of CO_2, 57 volumes per cent., and H, 40 volumes per cent.

Albumen was not affected by this microbe. The meat cultures remained clear, and the meat was undissolved.

IV. *Bacillus liquefaciens ilei.*

They are small and delicate bacilli; 2 to 2·3 μ long and 0·4 μ in diameter. They form no spores, grow quickly, and are motile. Examined in a glass cell they dart across the field of vision with great rapidity. They do not colour well with the usual pigments, best of all with methylene blue. At 15° C. they grow rapidly on gelatine plates. After two days the colonies are visible to the eye as small round points. They have a sharp clearly-defined margin, produced by a liquefaction of the gelatine surrounding the colony. Examined with a low power the colony has a brownish tint, and the periphery is not sharply defined. Surrounding it is a layer of liquefied gelatine (*v.* Plate X. fig. 4). In stabcultures a tube-like liquefaction of the gelatine takes place. The liquefied gelatine contains dull white flakes consisting of masses of bacteria. At the bottom of the tube there is a whitish deposit of bacteria. At the end of two weeks the gelatine is completely liquefied. In agar the bacteria form a greyish-white moist pellicle over the entire surface. They grow quickly in bouillon cultures, kept at 38° C. After 24 hours the bouillon is quite cloudy, and after 2 days a thin pellicle forms on the surface, which on shaking falls to the bottom of the tube. There is no distinctly putrefactive odour. Fresh sterile milk is not coagulated by this bacillus.

Dextrose is only decomposed to a small amount. The culture was pure, the fluid neutral and without smell. It contained 3·2 per cent. unchanged sugar. On distilling we obtained a small quantity of alcohol, too small to determine its nature. There were traces of a volatile fatty acid, probably acetic acid. From the ether extract a zinc salt was obtained, not enough, however, for a trustworthy analysis.

In the flask containing meat, about half of the meat was decomposed. The fluid had the smell of old cheese. The reaction was strongly alkaline, and on adding caustic soda much ammonia was developed. It contained, however, neither indol, skatol, or methylmercaptan. The bacillus had therefore an action on proteids, and is being further investigated at present in this laboratory.

V. *Bacterium ovale ilei.*

The bacteria are almost circular, and in appearance at times closely approaching micrococci. Numerous transition forms were seen up to distinctly bacillary forms. On gelatine plates

the colonies have a brownish tint, and are round or oval, with an irregular contour (*v.* Plate X. fig. 5). In "stabcultures" they grow on the surface of the gelatine as a flat greyish-white layer, like the head of a nail. Along the line of inoculation the growth is granular and dullish white; at the lower part large, isolated, and bead-like colonies are seen. They grow quickly in meat broth, and there is no putrefactive smell. They do not coagulate milk.

The culture in sugar was pure. The solution contained 1·3 per cent. unchanged sugar. On distilling we obtained 3·5 c.c. ethyl alcohol. There were traces of a volatile fatty acid, most probably acetic acid. The remainder, after adding oxalic acid, and distilling, was extracted with ether. From the ether extract we obtained 0·4 grm. of a zinc salt. It was the active paralactic acid—0·216 grm. lost at 110° C. 0·028 grm. in weight = 12·9 per cent. water, and left after combustion 0·063 grm. ZnO = 26·87 per cent. zinc.

The albumen was unaffected by this microbe.

VI. *Bacillus gracilis ilei.*

It is a slender rod bacillus, about five times as long as broad. The bacilli are generally in pairs and are motile (Plate X. fig. 6). No spores were observed. On gelatine plates they form yellowish-white round colonies with sharply-defined margins. In "stab-culture" the gelatine becomes covered with a thin delicate pellicle of a dull white colour. In the depth the growth is feeble. In bouillon they grow well at 38° C. Milk is coagulated after twenty hours.

The sugar culture was pure, and contained 2 per cent. unchanged sugar. The amount of alcohol was about 4 c.c., of which the greater part distilled over at 77° to 80° C. A small quantity distilled at a higher temperature, but the amount was not sufficient to determine its nature. Traces of a volatile fatty acid were found, from the qualitative tests most probably acetic acid. The ether extract yielded about 0·3 grm. of a zinc salt. It was paralactic acid—0·186 grm. lost at 110° C., 0·023 grms. in weight = 12·36 per cent., and left on combustion 0·054 grm. zinc oxide = 26·58 per cent. Zn.

The meat cultures remained unchanged.

VII. *The Bacterium probably identical with the Bacterium lactis aerogenes of Escherich.*

These bacteria have sharply-rounded ends, are single, or united in pairs or in groups. On gelatine plates they form superficially

white glistening colonies. The deeper colonies have a yellowish tinge, and are round (*v.* Plate X. fig. 7). In "stabcultures" the growth is bead-like. Superficially they form a porcelain-white flat growth. The growth in agar is similar. They grow well and rapidly in bouillon. They coagulate milk within 20 hours at 38° C., but first after four days at 15° C. Like the bacterium lactis aerogenes they are pathogenic for guinea-pigs—the animals died in 2 to 4 days.

The sugar culture was optically inactive, though it still reduced an alkaline solution of sulphate of copper. The sugar was therefore almost entirely decomposed. On distillation, a large quantity of alcohol was obtained—about 8 c.c. On rectifying, it distilled at 77° to 79° C., with the exception of a small remainder. Acetic acid was present in small amount. The silver salt contained 64·55 per cent. silver. The remainder contained much succinic acid. The crystals melted at 180° C. A zinc salt was also obtained, most probably of lactic acid. Its amount was not sufficient for analysis. Anaerobic cultures of the bacterium were made in sugar solutions. The air was replaced by CO_2. There was an active development of gas. According to an analysis made by Dr Frey, the gases were CO_2, 72·38 per cent., and Hydrogen, 27·61 per cent.

It will be necessary at this point to make some general observations regarding the bacteria found in other sections of the digestive canal. We will then state the conclusions our investigations have led us to with regard to the share the bacteria take in the decomposition of the food. No one now doubts that the entire digestive tract (mouth cavity, stomach, small and large intestine) constantly contains bacteria.

The investigations of Sucksdorff[1] show that their number can vary greatly, according to the diet and its method of preparation, *i.e.*, the degree of sterilisation that takes place in cooking.

Miller[2] has made detailed researches as to the bacteria contained in the mouth cavity. He isolated a large number of micro-organisms which have their chief seat in the mouth. He further found that five of these produced a considerable development of gas in fluids containing sugar. We will mention two of these—the *Micrococcus aerogenes* and the *Bacterium aerogenes*—since it was possible that they might be identical with forms isolated by us.

Amongst the three bacilli isolated by Raczynski[3] from the dog's stomach after meat diet, one, the *bacillus geniculatus*, may possibly be identical with our *bacillus liquefaciens ilei*.

[1] *Archiv f. Hygiene*, vol. iv., 1886.

[2] *D. Med. W.*, 1884, Nos. 36 and 38; 1886, No. 8; 1888, No. 30.

[3] *Centralblatt für Bakter.*, vol. vi. p. 112.

Escherich[1] found very varying forms in the meconium, and amongst these bacteria usually found in putrefying fluids. The bacteria he isolated were—

1. Bacteria with swollen ends; not isolated in pure culture.
2. Rod bacilli; perhaps identical with bac. subtilis.
3. A small streptococcus which liquefies gelatine, called by him the s. coli gracilis.
4. Bacterium coli commune; few in number.
5. A number of micrococcus forms.
6. A yeast fungus.

In "milk fæces," on the contrary, there was not such a variety of forms. Two species were most constantly present—the bacterium coli commune, and the bac. lactis aerogenes.

Three of the above forms were also found in the intestinal tract, and the fæces of Carnivora, viz.:—

1. The bact. coli commune, especially numerous in the lower part of the digestive tract, and in the stools of sucklings. 2. The bacterium lactis aerogenes. 3. The streptococcus coli gracilis, chiefly in meconium fæces.

As regards the microbes in the large intestine of Man, we will mention the researches of Bienstock[2] and W. Booker.[3] The first author isolated from human fæces four microbes, one of which he regarded as the special cause of the decomposition of proteids. The second author investigated chiefly the fæces of sucklings. He found the bact. coli commune in the normal "milk fæces." In diarrhœic discharges the number of the bact. coli commune lessens proportionally with the severity of the attack. In its place appears as predominating form a short rod bacterium resembling the bacterium lactis aerogenes.

Amongst the forms more particularly studied by us are *three* which might possibly be identical with some of the forms isolated by earlier observers from the intestinal contents. These are—

1. *Streptococcus liquefaciens ilei*, with the *strept. coli gracilis.*
2. *Bacterium Bischleri*, with the *bac. coli commune.*
3. The *bacterium* or short rod bacillus vii., with the *bact. lactis aerogenes.*

The *Streptococcus liquef. ilei* differs in two points from the streptococcus coli gracilis of Escherich. It liquefies the entire gelatine from above downwards, and is pathogenic for Guinea-

[1] *Fortschritte der Med.*, vol. iii.
[2] *Zeitschrift für Klin. Med.*, vol. viii.
[3] *Centralb. für Bakter.*, vol. v.

pigs. Escherich's microbe produces a funnel-shaped liquefaction of the gelatine, and is non-pathogenic for Guinea-pigs.

Microscopically, the *bacterium Bischleri* resembles the bact. coli commune. The former, however, forms from sugar the *in*active, the latter the *active* lactic acid. They are therefore not identical. For the same reason it is possible that the bacillus isolated by Gessner[1] from the human duodenum, and called by him bac. coli commune, may be identical with the bac. Bischleri.

As to the third microbe, it is most probably identical with the bact. lactis aerogenes. They are short and rod-like bacteria, about 2 μ long, and are slightly less in diameter. The size, however, is not constant; they are sometimes larger, at times more spherical in shape, and resembling cocci. They are facultative anærobic; coagulate milk within 24 hours at blood temperature, and in stabculture resemble closely the bac. lactis aerogenes. As already mentioned, they do not decompose albumen, but form out of sugar alcohol, succinic acid, acetic and lactic acids.

We did not find the Bienstock bacilli in the small intestine. It was therefore of interest to investigate the bacteria in the large intestine of the patient, who had not defæcated for two months. For this purpose the large intestine was washed out from the rectum with sterile salt solution. After a few minutes portions of the fluid flowing out of the *upper* end of the large intestine were collected for examination. Microscopically we could distinguish three species—

1. Streptococci, present in greatest number.
2. Short rod-bacilli, also numerous.
3. Slender bacilli, probably identical with Bienstock's bacillus. The number present was small.

The bouillon cultures soon acquired a putrid smell, and had a green fluorescent colour, both due to the streptococci. They also liquefied gelatine, which acquired a green fluorescent colour.

The short rod-bacillus in pure culture did not liquefy gelatine, and corresponded morphologically to the bac. coli commune.

On the plates also were bacilli, which produced in bouillon a putrid smell, but without fluorescence.

[1] *Centralb. für Bakter.*, vol. vi.

Two weeks later the experiments were repeated after the patient had received per rectum egg clysters. Microscopically, the appearance was much the same as in the former case, only the streptococci were relatively few in number. All samples from the large intestine developed in bouillon a disagreeable putrid odour, and the majority of the plate colonies consisted of a fluorescent putrefactive bacillus.

Our chemical and bacteriological investigations show that, under NORMAL conditions, the bacteria in the small intestine of Man do not, as a rule, decompose proteids, or do so to a very small degree.

The bacteria present in the normal small intestine decompose especially the carbohydrates, and the products of the decomposition are ethyl alcohol, the two lactic acids, acetic acid, and succinic acid.

These products were also isolated by us *directly* from the contents of the small intestine.

The proteids are decomposed in Man, in the *large* intestine, under formation of the well-known products, indol, &c.

Already Ewald, after his investigations on a patient with intestinal fistula, concluded that it was inadmissible to assume any other source for the indican and phenol than the *lower* part of the digestive canal. The same also applies to sulphuretted hydrogen and methylmercaptan.

In support of this statement we may mention the following fact. More than ten years ago experiments were made in the Berne Hospital with a view of testing the antiseptic value of the nitrate of bismuth. In the patients who died, and who had received bismuth, it was interesting to notice that the entire mucous membrane of the large intestine, from the ileo-cæcal valve downwards, had a dark velvet-like appearance, whilst the mucous membrane of the whole *small* intestine was merely reddened. Investigation showed that the blackening of the mucosa was due to sulphide of bismuth. The absence of the same in the ileum mucosa showed that there *no* H_2S was developed.

The general surveys made of the bacterial forms present in the small intestine, under normal and healthy conditions, show that these vary, even with a slight change in diet, or the way in which it is prepared.

There seem to be no bacterial forms that are specially bound to and constantly to be found in the small intestine, as, *e.g.*, seems to be the case with Leptothrix in the mouth cavity, and the bac. coli commune in the large intestine. One *characteristic* mark, however, for the bacteria of the small intestine is this—that by preference, so to say, they decompose carbohydrates and not proteids.

We cannot say with exactness how much of the sugar derivatives in the small intestine is due to the action of bacteria. This will depend on the following factor, viz., if those microbes which energetically split up sugar prevail amongst the others or not—*e.g.*, the streptococcus liquefaciens ilei, or the bacterium lactis aerogenes.

The organic acids formed out of the sugar are the causes of an increase in acidity of the chyme passing out of the stomach to such a degree that neither the alkali of the bile, nor that of the pancreatic juice, or of the entire mucosa of the small intestine, is sufficient to completely neutralise the food-mass.

An approximate notion of the amount of alkali furnished by the intestinal mucosa for the purpose of neutralising the acids, is furnished by the *ash*-analyses we made. These analyses were made not only after a proteid diet, but also after a diet in which carbohydrates predominated. The results are given in the table on p. 418. The intestinal contents, dried on the water-bath and then at 110° C., can be easily powdered. By heating on platinum an ash is left with a strongly alkaline reaction; on adding HCl, carbonic acid is given off.

The estimation of iron, silicic acid, alkaline earths and alkalies, was made as follows:—12·8828 grms. of the dry remainder after meat diet was carefully carbonised in a platinum dish, and heated till all organic matter disappeared. The soluble constituents of the ash were extracted with water, and filtered. The filtrate was evaporated in a platinum dish, and the remainder gently heated. Nitrate of ammonia was added to the remainder on the filter, which was then heated till a white ash resulted. This, after weighing, was added to the "soluble ash," and dissolved in HCl. The total amount of ash obtained was 8·33 per cent., of which 2·07 per cent. was soluble in water, and 6·26 per cent. insoluble. The usual analytical methods were employed for estimating the iron, silicic acid, and the bases.

The solution of the chlorine alkalies was repeatedly evaporated till no traces of baryta remained.

The chlorine, sulphuric and phosphoric acids, were estimated as follows:—30·9412 grms. of the dry remainder (equivalent to 2·5774 grms. ash) were dissolved in a 1 per cent. solution of nitric acid. The fluid was then filtered, the remainder on the filter washed till no further chlorine reaction was given by the filtrate. The filtrate + "wash-water" was then diluted to 800 c.c.: of this, 200 c.c. was

used each time for the estimation of the three acids. By estimating the acids by a moist method we avoided (1) any loss of chlorine, (2) any excess of sulphuric acid from the sulphur of the albumen, and (3) any excess of phosphoric acid from the phosphorus of the lecithin. The ash analyses of the intestinal contents, after a carbohydrate diet, were made in similar fashion. The following table gives the percentage amount of the different ash constituents :—

	After Proteid Diet.	*After Carbohydrate Diet.*
	[Ash in solid remainder =8·33 per cent.]	[Ash in solid remainder =8·6 per cent.]
	In 100 Parts Ash was found—	
CaO, . . .	29·58 per cent.	21·71 per cent.
MgO, . .	4·65 ,,	6·09 ,,
Na_2O, . .	31·53 ,,	30·94 ,,
K_2O, . . .	3·83 ,,	6·45 ,,
Fe_2O_3, . .	0·31 ,,	0·44 ,,
SiO, . . .	0·73 ,,	0·87 ,,
Cl, . . .	7·75 ,,	4·84 ,,
SO_3, . . .	1·22 ,,	0·47 ,,
P_2O_5, . .	14·46 ,,	10·68 ,,
Total, . .	94·06 ,,	82·49 ,,

The figures obtained are in several respects interesting. In both analyses the amount of the acids is much smaller than that of the bases. If we assume that in the first ash analysis all the chlorine is present as NaCl, the sulphuric acid as SO_4Na_2, and the phosphoric acid as PO_4HCa, then, in order to neutralise the mineral acids, 7·7 grms. Na_2O and 11·40 grms. CaO would be necessary. The remainder of the bases is united with organic acids, viz., 18·18 per cent. CaO, 4·65 per cent. MgO, 23·83 per cent. Na_2O, and 3·83 per cent. K_2O. From this it follows that 39·54 per cent. of the bases is combined with mineral acids, the remainder with organic acids.

If we make a similar calculation with regard to the mineral acids found after carbohydrate diet, the amount of alkalies necessary to neutralise them would be 4·54 grms. Na_2O, and 8·42 grms. CaO. The remainder of the bases united with CO_2 and organic acids would be 13·29 per cent. CaO, 6·09 per cent. MgO, 26·4 per cent. Na_2O, and 6·4 per cent. K_2O. From this it follows that 19·9 per cent. of the bases is combined with mineral acids, and the larger remainder with organic acids. In contrast to the salts formed with organic acids, the amount of the sodic chloride and of all the mineral salts is much less.

A very important and hitherto unregarded function of the intestinal mucosa is the supplying of alkali to the chyme. An

adequate neutralisation of the acid intestinal contents is of essential importance for the normal digestion in the small intestine. Should the mucous membrane furnish too little alkali, a hyperacidity of the intestinal contents must consequently ensue, whereby the separated mucin, instead of becoming mixed with the food-mass, is immediately precipitated on the intestinal mucous membrane. In the same manner also the bile acids would be precipitated. Digestion and absorption must thereby suffer. We have, as a matter of fact, observed that the more diarrhœic, semi-fluid intestinal contents contain the largest amount of sugar and acid—*vice versa*, an alkaline reaction of the intestinal contents would favour putrefactive changes.

Inasmuch as the mucosa furnishes the alkali as a carbonate, a portion of the CO_2 gas in the small intestine results from the neutralisation of the acid chyme. The remainder of the CO_2, as well as the hydrogen, result from the fermentation of the sugar.

It is the acids of the stomach and the small intestine which not only prevent the fermentative decomposition of albumens, but also limit the decomposition of carbohydrates. The earlier researches made prove this,[1] and also the more recent, especially made by us in this case. To bouillon we added lactic and acetic acid respectively, so that titrimetrically the fluid contained 1 per 1000 of each acid. It was then inoculated with the several bacteria isolated by us from the intestine. At 38° C. the fluid remained perfectly clear, and the growth of the microbes was completely suspended. After two days gelatine plate cultures were made, and on all colonies of the several bacteria grew. Thus the two acids in the above concentration did not kill the bacteria; they merely hindered their growth.

In apparent contradiction to this is the fact that numerous bacteria are to be found, not only in the intestine, where the acidity, as acetic acid, averages 1 per 1000, but also in the stomach, where the free HCl acid acts as an antiseptic. Experiments made on dogs with a strongly acid gastric juice proved that widely different bacteria (*e.g.*, micrococcus tetra-

[1] N. Sieber, *J. für Prakt. Chemie*, vol. xix.; and Thol, *Ueber den Einfluss Organ. Säuren auf Fäulniss u. Gährung*, Griefswald, 1885.

genus, st. aureus, and bacillus of rabbit septicæmia) passed through the stomach unscathed, and could be isolated again from the small intestine.[1] The reason for this is probably that the bacteria differ from one another in their susceptibility to the action of acids. In general those which decompose carbohydrates are more resistant than those which decompose proteids. Of those taken up with the food a number will certainly be destroyed in the stomach. The inimical action of the acid is also exerted in the whole length of the small intestine, so that during our investigations we never were able to isolate *putrefactive bacteria* from the intestinal contents. On the other hand, putrefactive bacteria were easily and constantly isolated by us from the large intestine of the same patient.

Probably only isolated spores of the bacteria which decompose proteids pass into the large intestine, where they settle down and develop.

It may be that *exceptionally* the contents of the large intestine have an acid reaction. Each time we examined the fæces of healthy and sick people the reaction was alkaline.

A second reason why isolated bacteria escape the inimical action of the acids is more of a mechanical nature. It can easily be demonstrated in animals, especially in the larger herbivora, *e.g.*, horses. If a horse, after being fed, is bled to death, and the stomach opened, the mucous membrane is found to have a strong acid reaction. The food lying on the mucous membrane is also acid, but at spots removed from the walls of the stomach, *e.g.*, towards the middle, the reaction is either neutral or alkaline. Notwithstanding the peristaltic action, all portions of the food-mass do not come into such intimate contact with the mucous membrane as to allow the acid of the same to kill the bacteria present in the separate particles of the food. It has further been shown by one of us that the bile and the bile acids have no marked antiseptic action.[2] The bacteria isolated by us grew well in gelatine containing 2 per cent. of bile.

On the 13th of November, *i.e.*, exactly six months after establishing the fistula, Professor Kocher once more united the small and large intestine. The patient made a good recovery; on the ninth day after the operation the first stool passed per rectum. The patient's condi-

[1] Macfadyen, *Jour. of Anat. and Phys.*, vol. xxi.

[2] Macfadyen, *Jour. of Anat. and Phys.*, vol. xxi.

tion continued to improve, and on the 19th December she was discharged as cured. During six months her large intestine was inactive. During this period it was completely shut off from the digestive process, excepting when a few peptone and egg clysters were administered, with a view of testing the absorption taking place from the large intestine.

It was of interest to ascertain how much of the food becomes digested and absorbed in the stomach and small intestine, and what share the large intestine takes in the process.

The patient received daily :—

In	260 grammes	Bread	16·2 grms.	albumen =	2·6 grms.	N. [1]
,,	100 ,,	Meat	20·8 ,,	,, =	3·33 ,,	N. [2]
,,	200 ,,	Barley gruel	3·21 ,,	,, =	0·514 ,,	N. [3]
,,	2 eggs	. . . . .	12·55 ,,	,, =	2·0 ,,	N. [4]
,,	20 grammes	Peptone	9·57 ,,	,, =	1·53 ,,	N. [5]
,,	100 ,,	Milk	3·41 ,,	,, =	0·547 ,,	N. [6]
,,	1050 ,,	Bouillon	5·0 ,,	,, =	0·081 ,,	N. [7]
		Total . .	70·74 ,,	,, =	10·602 ,,	N.

With the above diet, the amount of nitrogen in the dry remainder of the intestinal contents was 5·39 per cent. and 6·78 per cent., or on an average 6·08 per cent. The maximum amount of the semifluid discharge from the fistula was 550 grammes, with 4·9 per cent. of solid matter. The maximum amount of the consistent and porridge-like discharge was 232 grammes with 11·23 grms. of solids. The average amount of solids in 24 hours was 26·5 grms., containing 1·61 grm. N. = 10·06 grms. albumen. Inasmuch as the patient received in her daily diet 70·74 grms. albumen, it will be seen that only the seventh part of the albumen contained in her food (*i.e.*, exactly 14·25 per cent.) remained over for digestion and absorption in the large intestine, whilst 85·75 per cent. was absorbed in the stomach and small intestine. Carbohydrates were not absorbed to the same extent. They are decomposed largely in the large intestine, and also considerably by the bacteria of the digestive tract. Given in quantity they were discharged unchanged, as was proved by our experiments with mashed peas. Similar results were obtained by Bischoff and Voit in their experiments with dogs (*Die Gesetze der Ernährung des Fleischfressers*, Leipzig und Heidelberg, 1860, p. 290). A healthy dog, after a purely meat diet, discharged 27–40 grms. fæces in 24 hours in which were contained about 12·9 grms. solid remainder,

[1] König, *Die menschlichen Nahrungsmittel.*

[2] Estimated by us in the meat.

[3] Here also we estimated the amount of N., and calculated as albumen by multiplying with the co-efficient 6·25.

[4] One egg weighs 50 grammes. Albumen and N. were estimated according to König, p. 178, *l.c.*

[5] According to Pouchet's analyses of Kemmerich's peptone.

[6] König, *l.c.*, p. 203.

[7] The bouillon contained 2·2 per cent. solid remainder, and 0·162 per cent. N. Probably only half of the N. found therein is contained in the albumen and peptone ; the "meat bases" contain the rest. Therefore 0·081 grm. N. = 5·0 grm. albumen.

although the amount of meat given varied from 500 to 2500 grammes. The fæces were blackish, of the consistence of pitch, or solid, and were only discharged at intervals of several days. On the other hand, the fæces, after a diet of bread, were discharged at least once daily, and the amount was larger. The amount of solids corresponded to one-sixth or one-eighth of the bread consumed.

Bischoff and Voit found in their first experiments, that after giving the dog 857 grammes bread daily (containing 460 grms. solids), 377 grammes fæces were discharged, in which were 76 grammes of solids. This corresponds to 16·6 grammes fæces for every 100 grms. of bread. The fæces after a bread diet are yellowish-brown, friable, strongly acid, and are coloured deep blue by iodine.

If the percentage composition of the fæces after a bread diet be compared with that of bread, it will be found that the fæces consist almost entirely of unchanged bread, which the digestive apparatus is not able to assimilate. The percentage composition of the fæces after a meat diet on the other hand differ greatly. This will be made clear by the following table:—

	Bread.	Bread Fæces.	Meat.	Meat Fæces.
C.	45·51 per cent.	47·39 per cent.	51·95 per cent.	43·44 per cent.
H.	6·45 ,,	6·59 ,,	7·18 ,,	6·47 ,,
N.	2·39 ,,	2·92 ,,	14·11 ,,	6·50 ,,
O.	41·63 ,,	36·08 ,,	21·37 ,,	13·58 ,,
Salts.	4·12 ,,	7·02 ,,	5·39 ,,	30·01 ,,

By administering clysters to our patient per rectum we attempted to determine how much of the nutriment is retained and absorbed in the large intestine. We used for the injection Kemmerich's peptone and the contents of eggs beaten up in physiological salt solution. It was important to regulate the amount of fluid injected. When a large quantity was used a considerable portion was lost through the ileo-cæcal fistula. 100 grms. peptone were dissolved in 100 c.c. water, and injected into the rectum in two portions on the 28th of June. A portion was discharged from the fistula. Six hours after the injection 30 grms. of fæces were discharged, having a distinct odour and containing numerous triple phosphate crystals. The injections were repeated, the patient receiving this time only 80 grms. peptone dissolved in 80 c.c. of physiological salt solution. It was injected in two portions, an interval of four hours elapsing between the injections. In this case the *whole* of the peptone was retained, and during several days there was no discharge from the rectum. Five eggs were next mixed with 0·6 per cent. salt solution, and the whole diluted to 250 grms. This mixture was injected into the rectum in three portions on the 19th of July. Here also there was no discharge of the injected fluid from the fistula, nor from the anus. Thus 30 to 40 grms. of albumen were retained, and absorbed in the large intestine.

It is some years since Pasteur[1] raised the question as to the indispensability of bacteria in the digestive process. Our researches seem to negative the view that they take an active and necessary share in the decomposition of the food in the intestine of man. Pasteur communicated to the Paris Academy researches made by E. Duclaux with seeds sown in a sterilised soil. The soil contained neither nitrates nor nitrites and no ammonia, but was soaked with sterile milk, cane sugar, or starch-paste. The seeds received thus, instead of the usual simple carbon and nitrogen compounds, complex organic bodies as above. After one to two months the milk was still unchanged. It remained uncoagulated, and the casein was still precipitable by acids. The seeds behaved exactly as in the experiments of Boussingault with distilled water. Their dry weight became less and less the longer they remained in such a soil. The results with sugar and starch were similar. Duclaux concluded, and rightly, that plant life and growth are only possible when micro-organisms are present in the soil. The microbes break up the complex constituents of the mould into the simpler compounds, such as CO_2, H_2O, NH_3; nitric and nitrous acids, which can be utilised by the growing plant.

Pasteur added the following observations:—

"It would be highly interesting to feed a young animal from its birth on pure food constituents, *i.e.*, a nutriment artificially and entirely freed from all microbes. Without wishing to make a positive statement, I will not conceal the fact that if I had time to undertake the experiments I would do so with the preconceived opinion that life under such conditions would be impossible. Should such experiments prove to be capable of simplification, one would then, perhaps, be able to investigate the influence on digestion of the systematic addition of one or other of the different microbes to the food. The hen's egg would present the least difficulty, and be most suitable for such experiments. Before the chicken was hatched it would be necessary to clean the egg very carefully, and to place the hatched chicken at once in a space free from all bacteria, into which one could introduce pure air and pure food (water, milk, grain). Whether the results were positive or negative, the carrying out of the experiments would be of the greatest interest."

The opinion has already been expressed by one of us[2] that no one would probably question the proposition, "No plant-life in

[1] *Comptes rendus*, F. 100, p. 66.

[2] *Archiv für Exper. Pathol. u. Pharmak.*, vol. xx., 1885.

Nature *without* bacterial life." But, on the other hand, Pasteur's preconceived opinion, as stated above, is most likely an erroneous one, at least as regards vertebrate animals.

We have seen that, when the acid chyme passes from the stomach into the intestine, it becomes neither neutral nor alkaline, but preserves its acid reaction down to the ileocæcal valve. As a result of the acid reaction, a decomposition of proteids by bacteria does not take place, or at most inconstantly and to a hardly perceptible degree. And not only that, the action of the pancreatic juice on proteids is weakened by the acid reaction of the intestinal contents. The products so easily obtained outside the body by the action of trypsin on proteids (leucin and tyrosin) were *not* to be found in the small intestine. Further, the decomposition of the food-mass by bacteria is confined to the carbohydrates. The products formed by the bacteria from sugar are—the two lactic acids, acetic acid, succinic acid, ethyl alcohol, carbonic acid, and hydrogen. The ash analyses we made prove that an important function of the small mucosa of the intestine is to supply constantly alkaline carbonates for the purpose of neutralising the acids produced by the fermentation of the sugar. No one can suppose that these fermentation products are necessary for the maintenance of our life. It is much more to be regarded as a loss that a portion of the dextrose formed by the pancreatic enzyme out of starch is not absorbed, but serves as nutriment for the parasitic bacteria of the digestive tract.

It will be unwelcome news for some that a considerable amount of alcohol is produced in our bodies, not only in the small, but also in the large intestine, in the latter by the bacterium coli commune. The possible decomposition of the fats by the bacteria does not need to be considered here. It has already been shown by one of us[1] that the presence of bacteria does not materially affect the disintegration of fat in the intestine. The excellent researches of Immanuel Munk[2] have proved that about 90 per cent. of the food fats are absorbed as neutral fats, and that free fatty acids are already transformed into neutral fats in the walls of the intestine.

[1] *Archiv f. Exper. Pathol. u. Pharm.*, vol. xx. p. 374.

[2] *Virchow's Archiv*, vol. xxv. pp. 407–467.

With Munk's research before us, we felt it was hardly necessary to undertake an exhaustive inquiry into the composition of the fats in the small intestine of our patient.

During six months our patient lived with her large intestine completely cut off from the digestive process. During that time she gained in weight, and, as will be seen from the table giving the daily amount of urea excreted, the exchange of nitrogen increased constantly.

The emaciated body of the patient first of all retained a certain amount of the proteids, and it was only gradually that the nitrogen excreted in the urine came to correspond to the amount of nitrogen absorbed with the food.

Any considerable decomposition of the food by bacteria *first* takes place in the large intestine, and in our patient it was shut off from the rest of the digestive tract. It follows that the digestive juices alone, *without* the co-operation of bacteria, are able to prepare the constituents of our food for absorption, and to furnish the necessary material for the conservation of life.

The fermentation products of proteids in the large intestine are chiefly indol, skatol, phenol, lactic acid, volatile fatty acids, aromatic acids, organic bases and ammonia; the gases are carbonic acid, hydrogen, methan, sulphuretted hydrogen, and methylmercaptan. It is easy to see that none of these products are foodstuffs.

The organism has no need of these products; on the contrary they are injurious and burdensome to it when produced in excess in the intestine.

The facts that we consider to have proved with regard to man, may well also apply to other vertebrates. But here also the conditions may, in certain cases, be more complicated, *e.g.*, in herbivorous animals, and especially the ruminants where already in the first stomach a fermentation of the food takes place, and seems in this case to argue in favour of the necessary co-operation of bacteria.

[TABLE

Table showing the amount of Urea excreted by the patient, M. Spycher, from 15*th June to* 2*nd August* 1890,

(The Urea was estimated by Hüfner's method.)

Date.	Urine in 24 hours.	Reaction.	Specific Gravity.	Urea in per cent.	Urea in 24 hours.
	cc.			Per cent.	Grammes.
15th June . .	1260	Acid	1012	0·67	8·51
16th ,, . .	1100	,,	1011	0·736	8·09
17th ,, . .	a portion lost	,,	1010	0·916	...
19th ,, . .	1510	,,	1010	0·906	13·68
20th ,, . .	830	,,	1017	1·564	12·981
21st ,, . .	1010	,,	1010	0·85	8·58
22nd ,, . .	1220	,,	1013	1·081	13·18
23rd ,, . .	1240	,,	1012	1·002	12·42
24th ,, . .	1730	,,	1010	0·73	12·62
25th ,, . .	1150	,,	1015	1·19	12·07
26th ,, . .	1020	,,	1020	1·56	15·9
27th ,, . .	780	,,	1017	1·644	12·82
28th ,, . .	1375	,,	1015	1·394	19·23
29th ,, . .	1058	,,	1012	0·9234	9·76
30th ,, . .	1060	,,	1013	0·9234	9·78
1st July . .	782	,,	1021	1·64	14·62
2nd ,, . .	1225	,,	1015	1·199	14·68
3rd ,, . .	1450	,,	1015	1·21	17·59
4th ,, . .	1390	,,	1012	1·259	16·87
5th ,, . .	1000	,,	1013	1·035	10·35
6th ,, . .	1800	,,	1010	0·772	13·89
7th ,, . .	710	,,	1020	1·56	11·07
8th ,, . .	1485	,,	1013	1·12	16·63
9th ,, . .	1485	,,	1013	1·07	15·88
10th ,, . .	920	,,	1019	1·88	17·29
11th ,, . .	1200	,,	1014	1·22	14·73
12th ,, . .	1260	,,	1014	0·871	10·97
13th ,, . .	1080	,,	1012	1·028	17·27
14th ,, . .	1156	,,	1014	1·24	14·33
15th ,, . .	1005	,,	1017	1·624	16·32
16th ,, . .	955	,,	1017	1·87	16·87
17th ,, . .	1170	,,	1015	1·42	16·66
18th ,, . .	700	,,	1025	1·59	11·13
19th ,, . .	1005	,,	1020	1·59	15·97
20th ,, . .	1500	,,	1015	0·877	13·15
21st ,, . .	1080	,,	1013	1·038	11·24
22nd ,, . .	1460	,,	1014	1·19	17·40
23rd ,, . .	1225	,,	1018	1·57	19·23
24th ,, . .	2004	,,	1010	0·913	18·84
25th ,, . .	2030	,,	1010	0·786	15·95
26th ,, . .	1260	,,	1012	1·16	14·61
27th ,, . .	1630	,,	1013	1·09	17·76
28th ,, . .	985	,,	1013	1·33	13·10
29th ,, . .	1690	,,	1011	1·20	20·28
30th ,, . .	1020	,,	1014	1·36	13·91
31st ,, . .	1230	,,	1014	1·23	15·12
1st August .	1540	,,	1013	1·37	21·09
2nd ,, . .	890	,,	1020	2·088	18·58

EXPLANATION OF PLATE X.

Fig. 1. Intestinal contents after meat diet.

Fig. 2. Intestinal contents after carbohydrate diet. The starch grains treated with iodine.

Fig. 3. *a*, *Bacterium Bischleri*, deep colony in gelatine; *b*, *Bacillus Bischleri*.

Fig. 4. *Streptococcus liquefaciens ilei*.

Fig. 5. *a*, *Bacterium ilei*; *b*, the same as a colony in gelatine.

Fig. 6. *a*, *Bacillus liquefaciens ilei*; *b*, the same as a colony in gelatine.

Fig. 7. *a*, *Bacterium ovale ilei*; *b*, the same colonies in gelatine.

Fig. 8. *Bacillus gracilis ilei*.

Fig. 9. *a*, Bacterium, probably identical with *bacterium lactis aerogenes*; *b*, the same, a gelatine colony.

Fig. 1.

Fig. 2.

Fig. 3 a.

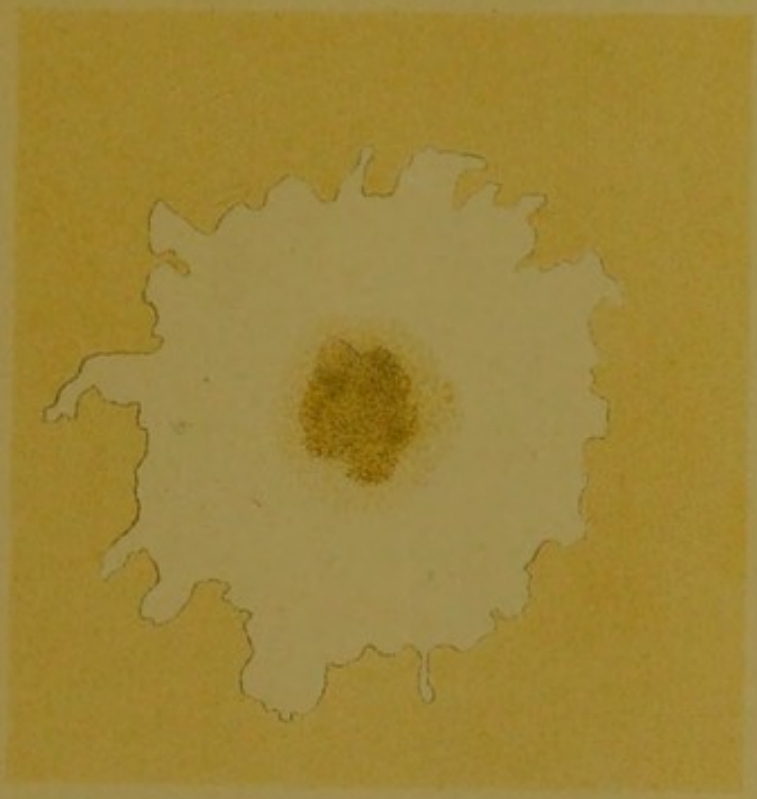

Fig. 1 a.

Fig. 1 b.

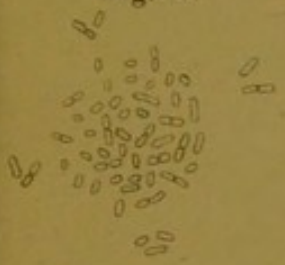

Fig. 2 b.

Fig. 2 a.

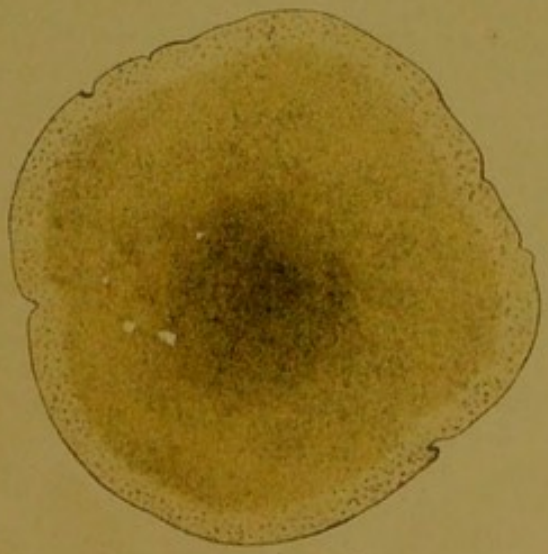

Fig. 5 a.

Fig. 3

Fig. 4 b.

Fig. 5 b.

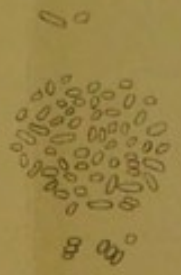

Fig. 6.

Fig. 7.