ALTERATIONS IN THE ENDOGENOUS ENTERIC BACTERIAL FLORA AND MICROBIC PERMEABILITY OF THE INTESTINAL WALL IN RELATION TO THE NUTRITIONAL AND METEOROLOGICAL CHANGES.

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(With 18 Charts.)

CONTENTS.

I. Introduction.

During the past few years, several workers in this laboratory have been investigating some changes in the bacterial flora, in the hydrogen-ion concentration, and in the permeability of the small intestine in relation to environmental changes. So far, we have studied, to a certain extent, changes produced by nutritional factors, such as acid-base content of food, also high protein and rich carbohydrate diets have been investigated. We have been interested in these biological changes in the small intestine in relation to climatic changes, such as "summer" and "winter" rooms for experimental work. Some few observations will be recorded upon sick animals, distemper and foreign protein intoxications. The author hopes in this communication to present some of the experimental results that we have recorded from time to time and briefly indicate their practical significance.

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II. ENDOGENOUS ENTERIC BACTERIAL FLORA.

(*a*) *Historical.*

Bienstock (1884) showed that the contents of the lumen of the upper half of the small intestine in laboratory animals was relatively free of bacteria, as compared to the lower part of the small intestine. Bacteria fed to such animals were not found to be added to the flora of this organ. Gessner (1889) found the upper segments of the intestinal tract in humans, after accidental death, to be relatively free of bacteria. These workers found only gram-positive cocci and assumed that the acidity of the stomach killed all bacteria ingested by mouth except the cocci found in the small intestine. Schuetz (1901) introduced "Vibrion Metchnikovi" directly into the duodenum of dogs, in this way eliminating the stomach, and found that they were killed before reaching the ileum. Rolly and Liebermeister (1905) reviewed this question, using *B. pyocyaneus* in rabbits and substantiated Schuetz's observations. These workers concluded that the healthy, intact mucosa of the small intestine was the most important factor in the "auto-sterilisation" mechanism of this part of the gastro-intestinal tract. The bile, pancreatic juice, intestinal juices, mucosal extracts, etc., all tested separately or in various combinations *in vitro*, had a growth promoting instead of a bactericidal action.

It would seem that the gastric acidity is one big factor in the destruction of ingested bacteria, but there is another mechanism in the lumen of the small intestine that is also responsible for the control of the endogenous bacterial flora in this region. Van der Ries (1925) reviews the literature and gives a summary of his work. This author used an ingenious apparatus for studying the enteric bacterial flora in man. A hollow cartridge that could be opened and closed by means of an electric magnet was attached to a long small rubber tube and passed into the stomach and intestinal tract by mouth in the usual manner. The position of the cartridge was determined by the distance from the mouth and by fluoroscopic examination. In this way, he was able to introduce bacteria, as well as chemicals, at any level of the intestinal tract and also to remove specimens for examination from any chosen segment. This author showed that bacteria introduced into the duodenum were destroyed before reaching the ileum in healthy persons. Van der Ries mentions an "auto-disinfection" of the upper part of the small intestine and not a "sterilisation" process, as it is never sterile, but has its obligate bacterial flora that is as constant as is the particular flora on any other part of the body surface.

(*b*) *Hydrogen-ion concentration.*

The hydrochloric acid secretion of the gastric mucosal glands acidifies the contents of this organ. The pancreas, liver and intestinal mucosa secrete alkaline fluids. These neutralise the gastric contents that is passed into the small intestine. It was thought that the contents of the small intestine was

alkaline in reaction. McClendon, Bissel, Lowe and Meyer (1920) reviewed the literature and reported many of their own observations. The upper part of the intestinal tract is slightly acid in pigs, puppies, dogs, cats, rabbits, also in man, infants as well as adults. Hahn, Klocman and Moro (1916) have pointed out that the infant's small intestine is acid in reaction from the duodenum to the middle or lower part of the jejunum.

(*c*) *Experimental.*

We attempted, in the beginning of our work upon the mechanism of control of the endogenous enteric bacterial flora, to establish fistulous openings at various levels of the small intestine and study the material obtained in this manner. We were soon convinced that such material could not be used for physiological studies. The higher up in the intestinal tract that one establishes such a fistulous opening, the quicker the animal becomes dehydrated from loss of fluids. There is also considerable digestion around such openings and these animals show, in addition to dehydration, some evidence of intoxication from the absorption of foreign proteins from their raw, digesting anterior abdominal wall. We were unable to avoid these difficulties, although many methods were tried in an effort to obtain a physiological preparation. After many attempts, by various procedures, we finally found that the best results could be obtained by attaching the small intestine to the anterior abdominal wall. Under aseptic operative procedures, the abdomen of an anaesthetised dog was opened by a median line incision. The desired part of the small intestine was found and attached to the anterior abdominal wall lateral to the line of incision. Before attachment, the peritoneal surface was cut and reflected for a distance of 10 cm. An elliptical section of abdominal muscle was cut out extending to the superficial fascia beneath the skin. This trough-like area was lined with the reflected peritoneal layer. The part of the small intestine selected was next placed in this depression and held in place by a few stitches through the serosa of the gut and adjacent tissue. The median line incision was closed in the usual manner. Traction on the mesentery, twisting and such changes in position of the segment of the intestine must be avoided. The development of this technic has allowed us to prepare ventral fixations of various portions of the small intestine without producing any evidence of obstruction. These animals are allowed to rest until the median incision is entirely healed. With a needle and syringe one can obtain specimens from the ventrally fixed portion of the gut or inject bacteria or chemicals into the lumen at will. In this way the stomach and most of the gastric variables that have heretofore entered into such work are eliminated, and the time factor of the entrance of material into the small intestine can be controlled (Arnold, 1926, 1). We have had twenty-five such animals for study at various times during the past four years, some have been observed for as long as three years. Table I gives the average results obtained by the use of our method. These results are the same as those obtained in experiments upon normal dogs.

Table I. *Bacterial flora and hydrogen-ion concentration of the small intestine (post digestive) of healthy dogs.*

Portion of small intestine	Bacterial flora	*p*H
Duodenum	Few gram-positive cocci	5·2–6·2
Upper jejunum	Few gram-positive cocci	5·5–6·5
Lower jejunum	Gram-positive cocci Few gram-positive bacilli Gram-negative bacilli	6·0–7·0
Ileum	Rich bacterial flora, faecal type	6·8–8·0

Arnold and Brody (1926, 2) injected alkaline phosphate buffered solutions into the ventrally fixed duodenum of normal dogs. This was followed by an ascension into the duodenum of the bacterial flora of the ileum, there was also a peripheral leucocytosis accompanying this reaction. There was no change in the rectal temperature of these animals. Dogs with "snuffles" and fever showed a faecal type of bacterial flora in the duodenum and a neutral reaction of the contents of the lumen. Fever produced by subcutaneous injection of staphylococcus vaccine caused a similar change in the endogenous bacterial flora and hydrogen-ion concentration of the upper part of the small intestine. The normal duodenal flora was not changed after tying off the pancreatic ducts. When the normal slightly acid reaction of the upper half of the intestinal tract is changed to a neutral or alkaline reaction, there is a change in the bacterial flora from a few gram-positive cocci to a rich growth of gram-negative bacilli and other faecal or large intestinal type of bacterial flora.

Arnold and Brody (1926, 4) extended the above-mentioned observations by obtaining caecal specimens through non-leaking appendicial fistulas in dogs. The self-disinfecting process in the upper part of the small intestine seemed to be dependent in some way upon the presence of acid buffered material within the lumen of this part of the tract. This reaction is maintained in the healthy animal by the normal gastric secretory functions. When neutral or alkaline buffered material enters the duodenum, this bactericidal power is inhibited and the bacteria in such material pass downwards and can be cultured from the contents of the caecum.

These results, obtained by feeding milk that had been made acid, or alkaline, were next extended to include external environmental temperature changes. Arnold and Brody (1927, 2) showed that when normal dogs are placed in warm rooms (temperature 90–95° F., humidity 90 per cent.) there is an interference with the normal self-disinfecting power of the intestinal tract. Bacteria ingested by mouth are passed on to the caecum in a viable state. The same animals, in ordinary temperature rooms, show the usual self-disinfecting power for ingested bacteria. Arnold (1927, 4) showed that bacteria injected into the ventrally fixed duodenum in dogs placed in the warm rooms soon appeared in the caecum in large numbers. These same animals, handled in a like manner, in ordinary temperature rooms destroyed

the bacteria before they reached the caecum. This seemed to show that the diminution in the gastric acidity in the high temperature environment was not the whole reason for the ingested bacteria appearing in large numbers in the caecum. Chart I shows graphically the relative auto-disinfecting power

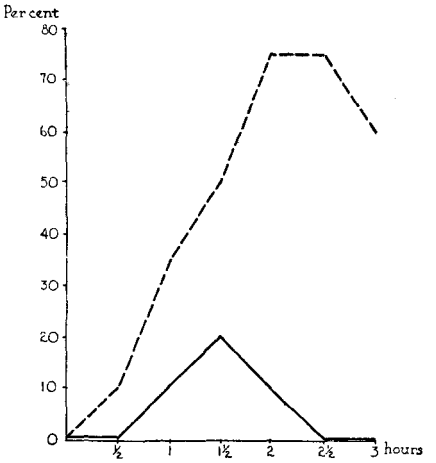

Chart I. Ordinate: percentage of *B. prodigiosus* appearing in caecum after intra-duodenal injection. Abscissa: time in half-hour intervals. Continuous line represents relative concentration of *B. prodigiosus* in ordinary temperature room. Broken line represents same in hot and humid room. Each graph is an average of nine experiments; three experiments were repeated using three different dogs.

of dogs with anteriorly fixed duodenum in warm and cool rooms. *B. prodigiosus* was injected directly into the duodenum in normal salt solution in both experiments.

The next six charts are reproduced to show the relationship between

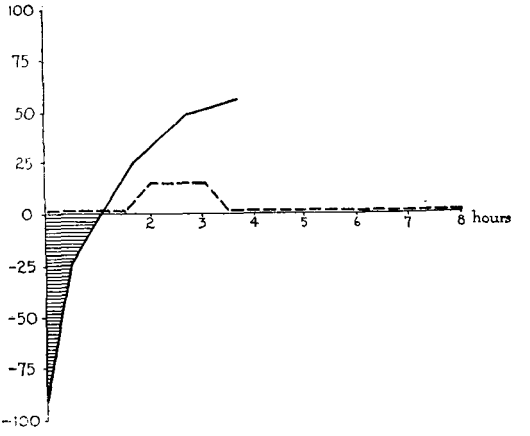

Chart II. 300 c.c. plain milk and one agar slant of *B. prodigiosus*. Continuous line represents acid titration of gastric contents, ordinate shows clinical units of acid-deficit or free acid. The wedge-shaped crossed-in area shows the relative acid-deficit of this meal. The broken line represents the percentage of the ingested *B. prodigiosus* that appears in the caecum, the ordinate showing percentage in this case. Abscissa represents time in hours after feeding.

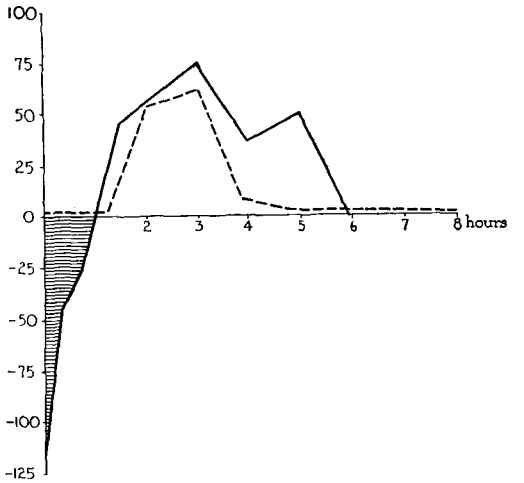

Chart III. 300 c.c. alkaline milk (*p*H 8·0) and one slant of *B. prodigiosus*. Continuous line represents acid titration of gastric contents. The ordinate shows clinical units of acid-deficit or free acid. The broken line represents the percentage of the test bacteria appearing in the caecum, the ordinate shows the relative percentage of bacteria as compared to the ingested material. The abscissa shows the time in hours after the meal was ingested.

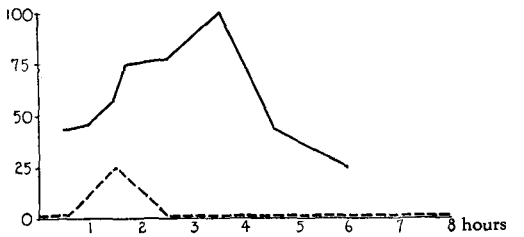

Chart IV. 250 grams of bread and one agar slant of *B. prodigiosus* was mixed well with 150 to 200 c.c. of 1 per cent. peptone water. The continuous line represents acid titration. The ordinate shows clinical units of free acid. Broken line represents the concentration of *B. prodigiosus* in the caecum, ordinate shows percentage of these bacteria in relation to the ingested mixture. The abscissa represents time in hours after the feeding of this mixture.

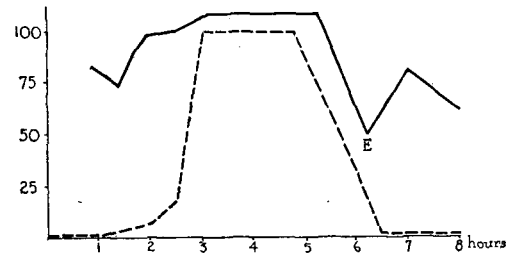

Chart V. One pound of lean Hamburger steak cooked in 500 c.c. of saline solution. One agar slant of *B. prodigiosus* was added before feeding. The continuous line represents the acid titration of the gastric contents, ordinate shows acidity in terms of clinical units. "*E*" is the time the stomach was emptied. The broken line represents the relative concentration of *B. prodigiosus* in the caecum, the ordinate the percentage of these bacteria in relation to the ingested material. The abscissa shows time in hours after the ingestion of the meal.

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gastric acidity, alkaline content of the food, high protein diet and fever upon the fate of ingested bacteria. Non-leaking gastric and caecal fistulae were made upon each dog. Some of these animals had the third part of the duodenum fixed to the anterior abdominal wall. Gastric and caecal specimens could be obtained at will. The contrast between Charts II and III, plain and alkalinized milk, needs no comment. Charts IV and V are interesting. Following a high protein diet there is an interference with the self-disinfecting power of

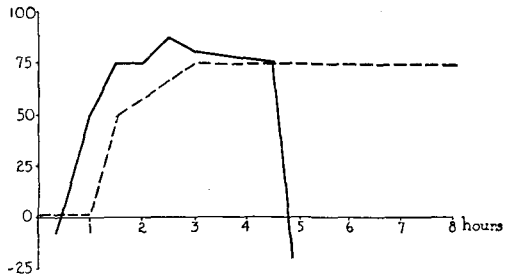

Chart VI. Same experiment as shown in graph in Chart IV, except animal was in a warm chamber (90–95° F. and 90 per cent. relative humidity).

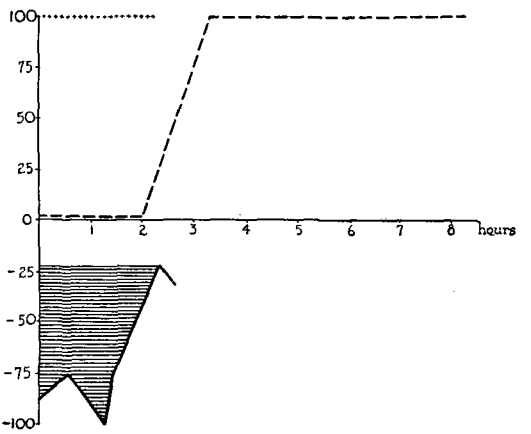

Chart VII. Dog with distemper. Rectal temperature 105·5° F. Copious purulent discharge from nose. 300 c.c. plain milk and one agar slant of *B. prodigiosus*. Ordinate in lower half represents acid-deficit in clinical units; in upper half of chart the ordinate shows percentage of *B. prodigiosus* in relation to ingested material. ..... line = gastric contents; – – – line = caecal contents. The continuous line with crossed-in area shows the acid-deficit of the gastric contents. Abscissa represents time in hours after ingestion of the meal.

the small intestine and this lasts for several hours. We have used several test bacteria during this work. *B. prodigiosus*, *B. pyocyaneus* and *B. enteritidis* have been added to the food and the percentage recovered in the caecum compared to that in the ingested material. In the high meat diets it was necessary to cook the meat in order to remove the bacteria in and on the raw meat before feeding, otherwise these bacteria were so numerous in the caecal specimen from the second to about the sixth hour after feeding that

our test organisms could not be detected with any degree of accuracy. Charts IV and VI should be compared; the same diet was given (bread moistened with broth) in these two series of experiments. Chart IV, dog was in ordinary temperature room; Chart VI, dog in hot temperature room (90–95° F. and 90 per cent. humidity). Chart VII shows an experiment upon a dog suffering from distemper. The rectal temperature was 105·5° at the time of the experiment. These experiments are cited from Arnold (1928, 3); the original can be consulted for further details.

III. PERMEABILITY OF THE INTESTINAL WALL FOR BACTERIA.

(*a*) *Historical.*

The epithelium of the gastro-intestinal tract covers a body surface that has not been studied to the same extent as the easier accessible epidermal covering of the body. Our main interest in the gastro-intestinal tract has been centred around its specialised digestive and absorptive functions. Nevertheless the single epithelial layer of the mucosa of the alimentary tract is a body covering and is in constant contact with a definite obligate bacterial flora. The type of bacterial flora is regulated under normal conditions to a certain definite zone or level of the alimentary tract. The oral, the upper intestinal and the lower intestinal zones have a rather constant obligate bacterial flora. The oral flora is not superimposed upon the duodenal and upper jejunal flora, but both are different in their composition. The bacterial flora of the faeces has been thoroughly studied by numerous observers. These types probably represent the bacterial life of the large intestine. The upper half of the small intestine is a specialised digestive and absorptive area. There is normally a scanty and rather constant flora in this region.

It is quite natural that the early bacteriologists were interested in the passage of bacteria through the intestinal wall into the body. The disease-producing bacteria were found to cause morphological changes in the intestinal mucosa and were therefore considered to have invaded the body through a broken or changed epithelial surface. The non-pathogenic bacteria were seldom found in the blood stream or in the internal organs after they had been fed by mouth to experimental animals. This led to the conclusion that the healthy intact mucous membrane of the alimentary canal did not allow bacteria to pass through into the body. The majority of investigators have examined the systemic blood stream in the living animal for the presence of bacteria that had been introduced into the gastro-intestinal tract. Many experiments were carried out upon animals killed after a variable period of time after feeding and the internal organs cultured to detect the presence of the ingested bacteria. The phagocytic power of the whole splanchnic area was tested along with many other body functions in such experiments. Arnold has recently reviewed the literature. Neisser (1896) cannulated the thoracic duct in dogs and could not find ingested bacteria in the lymph three

to four hours after a high protein and fat meal. Thiele and Embleton (1913) injected *B. mycoides* into the duodenum, jejunum and ileum of cats under ether anaesthesia and cultured the lymph collected from the thoracic duct. These bacteria were suspended in saline and in cream. Five-minute samples of lymph were cultured for as long as four hours. The micro-organisms did not appear in the lymph and they were not found in the organs of the cats at the completion of the experiment.

Before giving our experimental evidence of permeability of the intestinal wall for bacteria, it is necessary to first discuss certain recent contributions to the subject of adaptation of the body to external stimuli. This work has been a gradual outgrowth of a study of the mechanism of non-specific protein reactions (Petersen 1922, Weichardt 1926). There is a tendency for the vaso-motor reactions of the superficial or peripheral areas of the body (skin, subcutaneous tissue and skeletal muscles) to be in a state of equilibrium with the same vascular tonus of the internal or splanchnic areas (liver, spleen and gastro-intestinal tract). When the peripheral area of the body has an increased blood supply, that is, vaso-dilatation of the skin capillaries, there is a diminished blood supply (vaso-constriction) in the splanchnic region. The opposite is also true, that is, a vaso-dilatation in the splanchnic region is balanced or accompanied by a vaso-constriction of the peripheral region. With increase in the blood supply to an area there is an increase in the physiological activity of cells of such an area. If this takes place in the splanchnic region, there is an increase in the gastric secretion (Mueller and Petersen, 1926, 2, 1927, 1; Arquin, 1928), and an increase in the amount of bile secreted by the liver (Petersen and Mueller, 1927; Mueller and Kast, 1928). The liver and splanchnic region show an increase in permeability and other activities as measured by quantitative analysis of the thoracic duct lymph (Petersen, Milles and Mueller, 1928); there is also an increase in the concentration of leucocytes per unit volume of blood in the splanchnic vessels during this stage of activity (Mueller, 1926). The peripheral area of the body has an opposite reaction, that is, vaso-constriction, leucopenia, diminished permeability, etc.

When the peripheral or skin area of the body has an increased activity above that in the normal basal metabolic condition, then there is a peripheral leucocytosis, increased permeability (Mueller and Petersen, 1927, 1; Petersen and Oettingen, 1927), and increase in the activity of the skin glands. In this instance there is always a diminished activity in the internal or splanchnic region, that is a leucopenia, and diminished permeability as determined by thoracic duct lymph analysis. These workers have also followed the changes in the acid-base equilibrium of the thoracic duct lymph and systemic blood stream during these changes in the periphero-splanchnic equilibrium. It must be borne in mind that these experiments cover sudden and acute changes in this tonus reaction between the superficial and internal body areas. The intensity of the disturbance can be controlled at will, but the experiment is of relatively short duration (up to twenty-four hours' time).

This balance between the peripheral and splanchnic regions of the body has been found to be present throughout life. Only a few of the many literature references have been indicated in the above brief outline. The para-sympathetic nervous system has been associated with increased vascular supply and increased glandular activity; the sympathetic nervous system has been associated with the opposite reaction. Hence this periphero-splanchnic balance is often referred to in recent literature as the para-sympathetic and sympathetic nervous equilibrium. This is confusing from the anatomical standpoint, as well as from a certain pharmacological standpoint. But when the organism is looked at and examined as a whole in its mechanism of adaptation to external stimuli, the conception of Petersen and his co-workers of the periphero-splanchnic balance is evident.

Arnold and Brody (1926, 2) found that alkalinisation of the duodenum is accompanied by a peripheral leucocytosis. This reaction takes place within thirty minutes. The change in the duodenal flora to a faecal type, and in the hydrogen-ion concentration toward alkalinity, is accompanied by a change in the periphero-splanchnic balance. This was the first evidence we found that might indicate that we were dealing with this phenomenon. After presenting our experimental evidence on microbic permeability of the gut wall, we will attempt to correlate and discuss the whole question of the biological control of the endogenous bacterial flora, the acid-base equilibrium within the lumen of the upper half of the small intestine and the permeability of the wall of this part of the alimentary tract as a whole in relation to the periphero-splanchnic physiological balance.

(*b*) *Experimental.*

A cannula was placed in the thoracic duct in dogs under local anaesthetic. Lymph was collected aseptically for five minutes. Then the duodenum was exposed through a median line incision in the anterior abdominal wall under local anaesthesia. Bacteria suspended in various solutions were injected directly into the duodenum. This was done with a short needle and care was taken not to injure the mucosa. The duodenum was replaced in the abdomen and the small incision closed. The lymph was collected continuously from the cannula and divided each five minutes into separate sterile test tubes. One-fourth of a c.c. was plated out on agar and incubated for twenty-four hours. Forty c.c. of solution were used for duodenal injection; this was put in slowly to avoid distention. The alkaline solution was phosphate buffered solution, *p*H 8·0, the neutral was normal salt solution. The protein was fresh egg-white and dog serum; each was added to make a 10 per cent. concentration in the solutions used. The dog bile was used in the same concentration. *B. prodigiosus*, *B. coli* and *B. typhosus* have been the only bacteria used by us so far in this work. Table II represents an average of five dogs for each experiment and summarises our results so far.

Alkalinised egg-white placed in the duodenum of a dog allows many

Table II.

Solution injected with bacteria	Average number of bacteria in lymph per c.c.	Time of appearance after injection
Alkaline sol. and egg-white	500 to 1000	First 5 minutes lasting for 30 min.
Neutral sol. and egg-white	None	—
Alkaline sol.	None	—
Neutral sol.	None	—
Alkaline sol. and bile	50 to 100	First 5 minutes lasting for 20 min.
Neutral sol. and bile	3 to 5	During first 30 minutes
Alkaline sol. and dog's serum	None	—
Neutral sol. and dog's serum	None	—

suspended bacteria to pass into the lymph stream. Alkalinised bile has the same effect, but not so many bacteria appear in the thoracic duct lymph. There are a few bacteria which pass into the lymph stream when neutral bile is injected into the duodenum under the conditions of our experiment. From these experiments we think that the bacteria found in the lungs in dogs after oral ingestion (Ficker, 1904, 1905, 1906) were not due to aspirated bacteria from the mouth after feeding. The bacteria that pass into the lymph stream through the mucosa of the intestinal tract are carried to the venous system; and the first capillary bed these micro-organisms encounter would be in the pulmonary system. Neisser (1896) looked for ingested bacteria in the thoracic duct lymph three hours after feeding. In our experiments, all the bacteria have disappeared after one hour, usually after thirty minutes from the time they were injected. Thiele and Embleton (1913) used the cat and placed bacteria in the small intestine in normal salt solution and could not demonstrate the passage of the test bacteria in the lymph collected from the thoracic duct. We have substantiated a large part of their experiments. These workers used an ether anaesthetic; our work was done with local anaesthesia. We have not experimented with intestinal permeability under general anaesthesia, but we have been unable to demonstrate a change in the distribution of leucocytes in the peripheral circulation with alkalinisation of the duodenum when the dog is under ether. This can be demonstrated in the majority of dogs when local anaesthesia is used. Even under these conditions the autonomic nervous system changes cannot be demonstrated as well as with the use of a more physiological preparation. These experiments have been reported by Arnold and Brody (1928, 1), Arnold (1928, 3). We have made in all twenty-seven experiments in which the number of white blood cells per cubic millimetre of blood obtained from the ear of dogs was determined, during the course of the experiments in which the thoracic duct lymph was collected after intra-duodenal injections. As mentioned above, local anaesthesia was used in the experiments. Five charts, VIII to XII, are cited here as illustrating typical reactions. The change in the distribution of leucocytes is one of the reactions that accompany alterations in the periphero-splanchnic equilibrium (Petersen and Mueller, 1927). Our experiments tend to show that the microbic permeability of the wall of the intestinal tract is associated with a sudden change

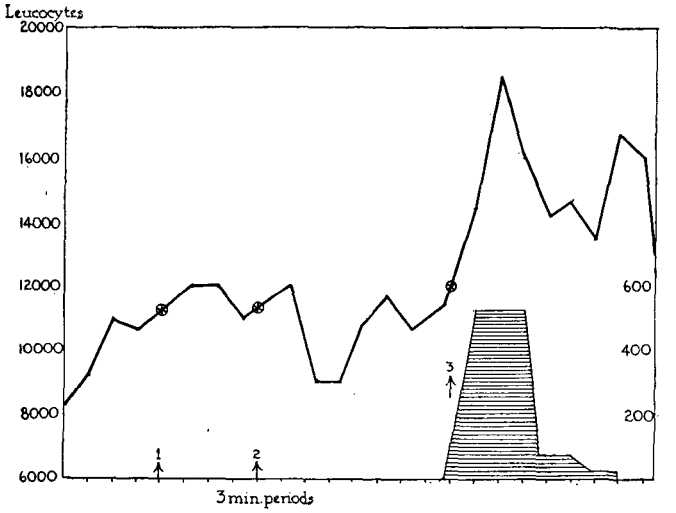

Chart VIII. Ordinate: number of white blood cells per cu. mm. of blood from ear of dog (left margin). Number of *B. prodigiosus* per c.c. of lymph collected from thoracic duct after intra-duodenal injection (right margin). Abscissa: time in three-minute intervals. Continuous line represents number of leucocytes. Ruled area represents number of *B. prodigiosus* per c.c. of thoracic duct lymph. 1. Time peritoneum was cut. 2. Time 20 c.c. saline plus one agar slant of *B. prodigiosus* was injected into lumen of duodenum. 3. Time 10 c.c. Na_2HPO_4 solution (*p*H 8·2) plus one fresh egg-white and one agar slant of *B. prodigiosus* was injected into the duodenum.

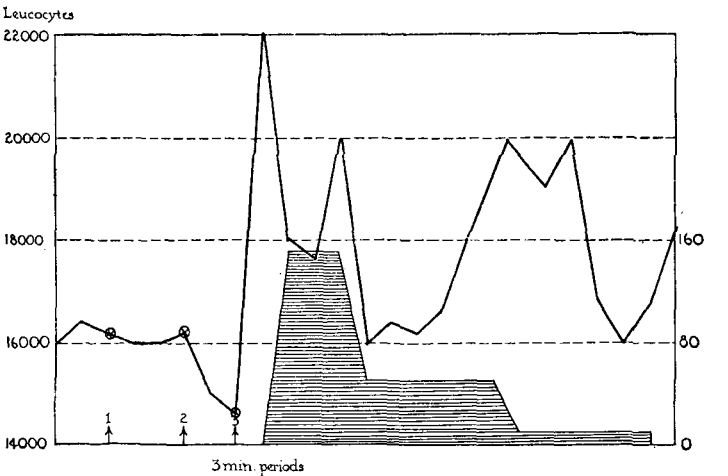

Chart IX. Ordinate: number of white blood cells per cu. mm. of blood from ear of dog (left margin). Number of bacteria per c.c. of lymph collected from thoracic duct after intra-duodenal injection (right margin). Abscissa: time in three-minute intervals. 1. Time peritoneum was cut. 2. Time 20 c.c. saline plus one agar slant of *B. typhosus* was injected into lumen of duodenum. 3. Time 20 c.c. Na_2HPO_4 (*p*H 8·2) plus one agar slant of *B. typhosus* was injected into duodenum. Continuous line represents leucocytes. Ruled area represents number of *B. typhosus* per c.c. in thoracic duct lymph.

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in the periphero-splanchnic balance. The reaction is usually one of peripheral, para-sympathetic or skin over-activity, but the opposite is sometimes the case. We were unable to demonstrate viable bacteria in the thoracic duct lymph in those animals in which there was a stable periphero-splanchnic

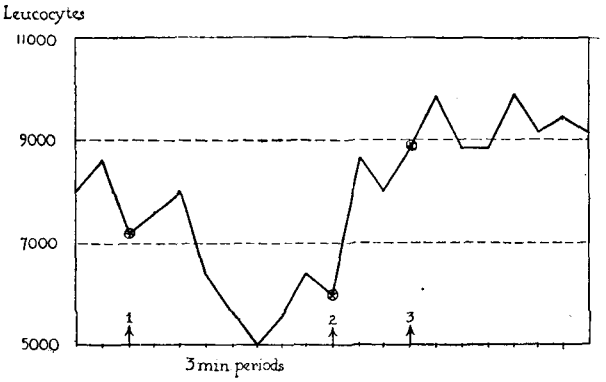

Chart X. Ordinate: number of white blood cells per cu. mm. of blood from ear of dog. Abscissa: time in three-minute intervals. Continuous line represents number of leucocytes. 1. Time thoracic duct was cut (dog was restless). 2. Time 20 c.c. saline plus *B. prodigiosus* was injected into lumen of duodenum. 3. Time 20 c.c. Na_2HPO_4 solution (*p*H 8·2) and 0·5 gm. desiccated ox-bile with *B. prodigiosus* were injected into duodenum. No bacteria appeared in the thoracic duct lymph.

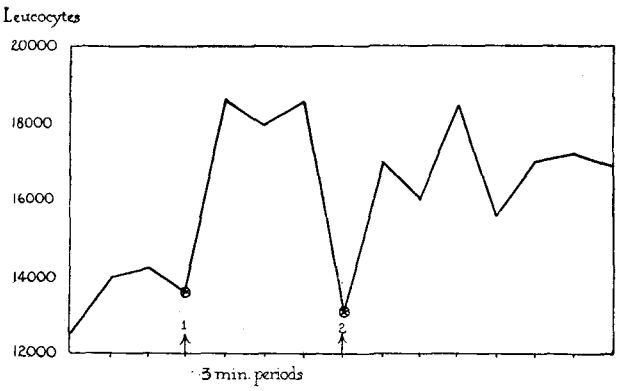

Chart XI. Ordinate: number of white blood cells per cu. mm. of blood from ear of dog. Abscissa: time in three-minute intervals. Continuous line represents number of leucocytes. 1. Time peritoneum was cut. 2. Time 10 c.c. Na_2HPO_4 solution and one fresh egg-white with *B. typhosus* were injected into lumen of duodenum. No bacteria appeared in lymph collected from thoracic duct.

balance, as manifested by a lack of disturbance in the leucocytic distribution in the peripheral capillaries.

The gastric secretion acidifies the contents of this organ under ordinary conditions. The amount of proteolysis and other digestive functions within the stomach might be open to question. Free acid in the stomach denotes a complete saturation of the gastric contents with acid. When this material

passes into the duodenum, the free acid is rapidly neutralised by the bile and pancreatic juice. The bound acid is liberated and replaced with alkaline radicals slowly. This probably plays a major part in the slight excess of hydrogen over hydroxyl-ions in the upper half of the small intestine. This has been found in herbivorous, as well as carnivorous and omnivorous animals. The normal animal seems to have a simple cocci-like flora in that region of the upper intestinal tract that is slightly acid in reaction. If this reaction is changed to neutral or alkaline, we have found that this is accompanied by an ascension into the upper levels of the small intestine of the bacterial flora usually found

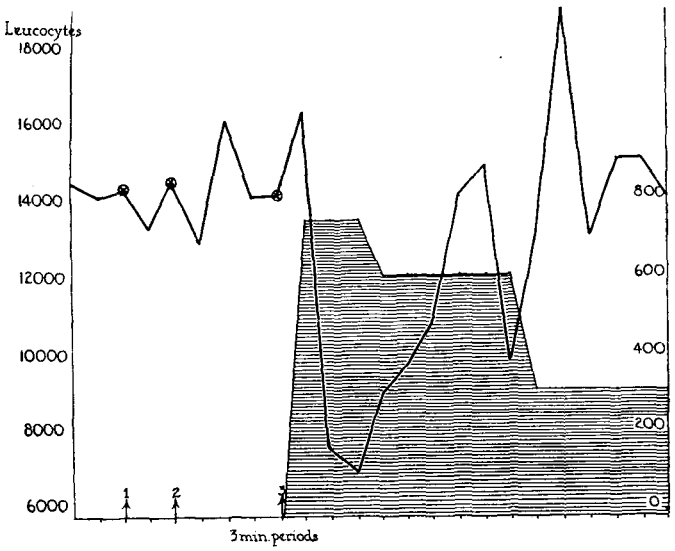

Chart XII. Ordinate: number of white blood cells per cu. mm. of blood from ear of dog (left margin). Number of *B. prodigiosus* per c.c. of lymph collected from thoracic duct after intra-duodenal injection (right margin). Abscissa: time in three-minute intervals. Continuous line represents number of leucocytes. Ruled area represents number of *B. prodigiosus* per c.c. of thoracic duct lymph. 1. Time peritoneum was cut. 2. Time 20 c.c. saline plus one agar slant of *B. prodigiosus* was injected into duodenum. 3. Time 10 c.c. Na_2HPO_4 solution (*p*H 8·2), one fresh egg-white and 0·5 gm. desiccated ox-bile plus *B. prodigiosus* were injected into duodenum.

in the lower alkaline-reacting segments. The bacteria placed in the duodenum under these conditions are not destroyed in the usual manner, but persist in a viable state for many hours or even days.

The glandular secretory activity of the stomach, liver, pancreas and intestinal mucosal glands function in a co-ordinated manner. The ingestion of food activates these glands. Carnot and Gruzewska (1925; 1926) have shown that bile, secreted during the period of active hydrochloric acid secretion by the stomach, has a greater alkali content than fasting bile or bile collected after placing acid in the stomach. This co-ordinated activity of the digestive glands takes place in the normal individual, that is, in the organism that has adapted itself to its environment. We have found that when this takes place, there is a slightly acid reaction in the upper half of the small intestine and

a relatively simple obligate bacterial flora in this region. Ascension of the faecal flora is prevented and ingested bacteria, or bacteria placed in the duodenum, are destroyed before reaching the lower segments of the small intestine. We consider this as a normal biological function of this part of the alimentary tract. The intestinal wall under these conditions is not permeable for bacteria, as determined by their presence in the thoracic duct lymph.

The inhibition of this bactericidal mechanism was most markedly demonstrable after a high protein meal and during a disturbance of the heat regulatory mechanism of the body, *i.e.* sudden elevation of external temperature and during fever. There is an increase in the oxidation by the cells of the body during these three different conditions. The specific dynamic action of protein is a well-known phenomenon. There is an increase in gastro-intestinal secretory and motor activity following a protein meal. The blood shows an increase in coagulability (Mills and Necheles, 1927). The capillaries of the skin change after a high protein diet, *i.e.* there are more capillaries visible in the same field and many of these show sacculated dilatations, as compared to the same area after a balanced diet (Gaensslen, 1927). We have observed an increase in the fluid content of the caecum during the period of active digestion following a meat meal in our animals. This fluid is reabsorbed in the colon, inasmuch as no increase in the frequency of defaecation has been noted. We have found that there is an apparent increase in concentration in the caecum of dogs of the d'Herelle's transmissible lytic principle after a high protein meal, and this lytic substance persists in the lower intestinal tract for twenty-four to forty-eight hours following a meat meal. These observations tend to show that there is a period of increased functional activity of the body following a protein meal. This period resembles in many ways a mild foreign protein intoxication. The increase in oxygen intake after a subcutaneous or intramuscular injection of a small amount of a foreign protein has been shown by Pollitzer and Stolz (1925), and also by Meyer (1927), to be comparable in intensity to that of a high protein meal. Mueller and Petersen (1927, 1) have come to the same conclusion, based upon their experimental evidence of the relationship of changes in the autonomic nervous system and gastric function. Toenniessen (1923) has emphasised the importance of the vegetative nervous system in maintaining the normal body temperature in poikilothermic animals. Changes in this nervous mechanism help such animals to maintain a constant internal temperature by regulating heat loss and heat conservation in different temperature environments.

Barbour and Hamilton (1925, 1; 1925, 2) have shown that the anhydremia observed when an animal is placed in a cold environment is due in a great part to fluids accumulating in the skin, subcutaneous tissue and muscles. Barbour, Dawson and Neuwirth (1925) found on the other hand that the hydremia observed in animals in hot environment was due to a mobilisation of water and salts from the tissue into the serum, the red blood-cells probably play a major part in this reaction. This favours the sweating process as an aid in

heat loss from the body. These observations show the complexity of the whole organic functional changes that are involved in our adaptation to environment.

When an animal is placed in an adverse environment, such as a warm and humid room for a short period of time, or given diluted heat-stable toxins of the Salmonella group of food-poisoning bacteria, there is an ascension of the bacterial flora of the ileum upwards. The first detectable change is noted in the lower jejunum, the contents of this part of the gut, instead of showing a transition between upper jejunum and ileum, has a typical ileal flora and the middle part of the jejunum represents the transitional zone. With further elevation in external temperature and humidity or a more potent gastro-intestinal irritation, these zones can be gradually seen to push cephalward until an ileal type of flora is against the pyloric sphincter, and even passes into the stomach under certain conditions. There is abundant evidence showing that the interference with the bactericidal power of the small intestine for exogenous bacteria is accompanied by a marked disturbance in the regulation of the endogenous bacterial flora of the lumen of this tract. The duodenum and upper portion of the jejunum are the last to lose their auto-disinfecting power.

IV. Relationship between gastric acidification, acid-base equilibrium in duodenum and intestinal self-disinfection.

Non-leaking gastric and appendicial fistulae were established in young healthy dogs. The third part of the duodenum was attached to the anterior abdominal wall so that material could be aspirated from, or injected into, the lumen by puncturing through the skin and gut wall with a needle attached to a syringe. A small amount of material was removed from the stomach at definite intervals and titrated in the usual manner for free acidity, acid-deficit, etc. *B. prodigiosus* was added to the material fed to these animals and the relative concentration of this organism was determined from caecal specimens removed at definite intervals by aspirating through the appendicial fistula. This indicated the relative bactericidal action of the gastro-intestinal tract above the caecum. Some material was removed from the lumen of the ventrally fixed duodenum by the method already mentioned. Chart XIII shows the results obtained after feeding 300 c.c. of plain milk. During the period of acid-deficit in the stomach (the first hour) the contents of the duodenum become more alkaline (pH 5·6 to 6·8). When the gastric contents are completely acidified and an excess of acid appears (free acid), the duodenal contents are more acid, or about what they were before feeding. There are relatively few bacteria reaching the caecum. Chart XIV records the same experiment, except that sodium hydroxide was added to the 300 c.c. of milk to give an hydrogen-ion concentration of pH 8·2. The acid-deficit is greater and lasts longer. The duodenal contents become more alkaline and many more bacteria reach the caecum. The auto-disinfecting power of the intestinal tract has been markedly inhibited. Chart XV records an experiment in which *B. dysen-*

teriae Shiga proteins were used. Bacteriophage was added to young broth cultures of Shiga bacilli and incubated until lysis or clearing had taken place. After passing through the Berkefeld filter to remove the few undissolved

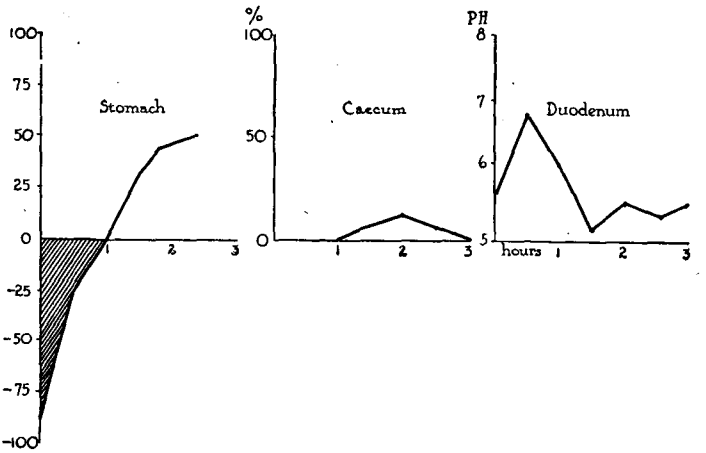

Chart XIII. Stomach: ordinate represents free acid and acid-deficit expressed in clinical units of material removed from gastric fistula. Ruled area shows relative degree of acid-deficit. 300 c.c. plain milk plus *B. prodigiosus* was fed. Caecum: ordinate shows the relative percentage of *B. prodigiosus* in material obtained from fistulous opening of this organ. Duodenum: ordinate shows *p*H of aspirated contents of duodenum. Abscissa of all graphs represents time in one hour periods.

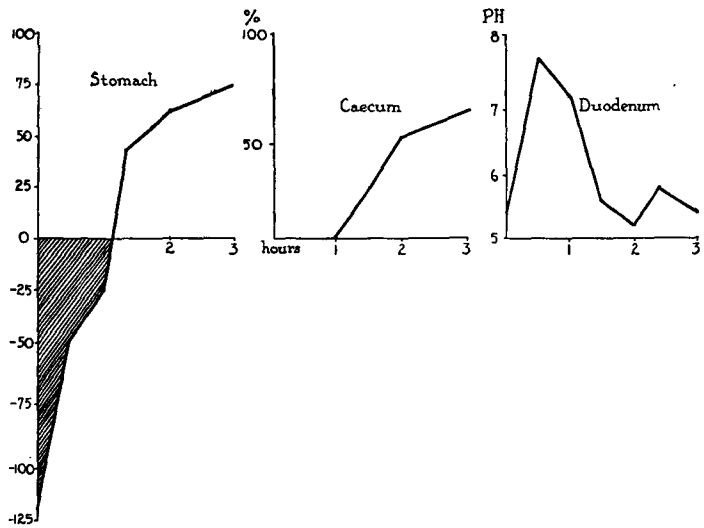

Chart XIV. Stomach: ordinate represents free acid and acid-deficit expressed in clinical units of material removed from gastric fistula. Ruled area shows relative degree of acid-deficit. 300 c.c. alkaline milk (*p*H 8·2) plus *B. prodigiosus* was fed. Caecum: ordinate shows the relative percentage of *B. prodigiosus* in material obtained from fistulous opening of this organ. Duodenum: ordinate shows *p*H of aspirated contents of duodenum. Abscissa of all graphs represents time in one hour periods.

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bacteria, 150 c.c. were given each animal by mouth. The subsequent course of the experiment was the same as the previous ones. These dogs were placed in a warm and humid room immediately after feeding. There was little if any gastric hydrochloric acid secretion, the acid-deficit persisted throughout the

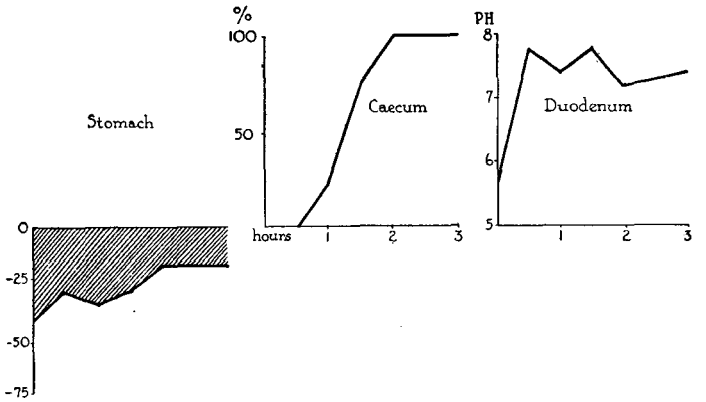

Chart XV. Stomach: ordinate represents acid-deficit (no free acid present in this experiment); 150 c.c. of bacteriophage-lysed *B. shiga* broth culture was fed. Caecum: ordinate shows the relative percentage of *B. prodigiosus* in material obtained from fistulous opening of this organ. Duodenum: ordinate shows *p*H of aspirated contents of duodenum. Abscissa of all graphs represents time in one hour periods.

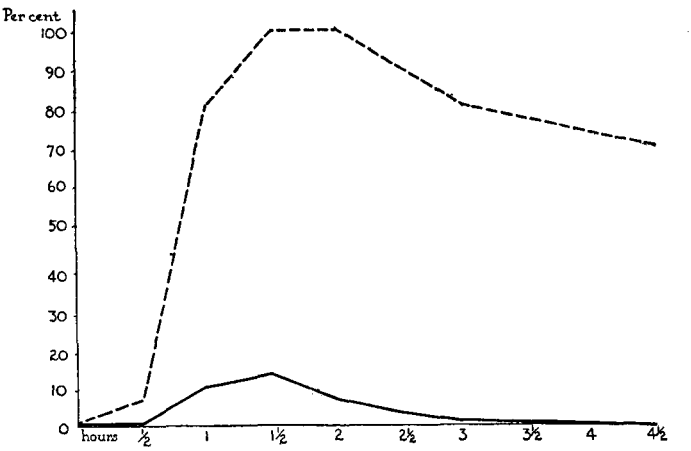

Chart XVI. Ordinate: relative percentage of *B. prodigiosus* present in the caecum. Abscissa: time in half-hour intervals. Continuous line shows the concentration of bacteria in per cent. after intra-duodenal injection in saline suspension. Broken line shows the same after injection of bacteria suspended in dibasic sodium phosphate (*p*H 8·0).

experiment. The duodenal contents became alkaline, the ingested bacteria appeared in the caecum in the same concentration as was present in the ingested material. All of the dogs had a diarrhoea, beginning between one and a half and two hours after feeding. The salivary secretions were increased, no vomiting occurring in any of the animals. The ingested bacteria were found

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in large numbers in the fluid faeces. These three groups of experiments show a certain relationship between the gastric acidity, hydrogen-ion concentration of the duodenum, and gastro-intestinal bactericidal action. The lack of acidity of the stomach might have been held to be responsible for these results. The next experiment excludes this explanation. Twenty c.c. of a normal saline suspension of one agar slant of *B. prodigiosus* was injected directly into the lumen of the ventrally fixed duodenum. The lower line on Chart XVI shows that relatively few of these bacteria reached the caecum and these soon disappeared from the contents of this organ. The same experiment was repeated, using 20 c.c. of alkaline phosphate buffered solution (*p*H 8·0) and *B. prodigiosus*. The interrupted upper line on Chart XVI shows that these bacteria reached the caecum within a short period of time and the concentration was the same as that injected into the duodenum. The bactericidal action of the gastric acidity could not play a part in this experiment.

V. Diarrhoea in infants.

The diarrhoea so often observed in summer, and following infections of the upper respiratory tract, internal ear, urinary tract, etc., in the infant may well be intimately associated with disturbances in the biological regulating mechanism in the gastro-intestinal tract. This question has been discussed in previous publications (Arnold, 1926, 3; 1927, 1). The normal infant does not concentrate hydrochloric acid in its stomach in sufficient amounts to cause the appearance of free acid. The contents are usually well acid-buffered when they pass into the small intestine, that is, *p*H 3·0 to 3·5. External temperature changes, or infections that will cause a diminution in the hydrochloric acid secretion will allow material to pass into the intestinal tract that does not contain sufficient bound acid to prevent alkalinisation of the small intestine in the presence of the alkaline digestive juices in this region. Moro (1916) described such disturbances as "endogenous infections." He found a faecal type of bacterial flora and an alkaline reaction in the upper part of the intestinal tract in such infants. The blood and various organs did not contain pathogenic bacteria. He suggested that this was caused by an absorption of toxic substances from the changed bacterial flora in the duodenum and upper jejunum, hence the term "endogenous infections." The use of acid-buffered food helps to prevent these gastro-intestinal disturbances. For addition to the milk, non-irritating organic acids or hydrochloric acid are commonly used. Findelstein's "Eiweiss-molke" is an excellent example of this principle. Ballot (1865) mentioned the use of buttermilk in Holland as an old method in infant feeding during the hot months of the year. The greater care that is taken in assisting the infant in the process of adaptation to its environment, the less will be the incidence of diarrhoea. Brownlee and Young (1922) have recently discussed these problems in detail.

The pasteurising or even boiling of cows' milk does not reduce its acid-demanding contents. Peters (1911) mentions that summer diarrhoea was not

appreciably influenced by boiling the milk used as infant food. Improvement in the sanitary quality of the milk supply has undoubtedly helped to reduce the incidence of diarrhoea in infants. In my opinion, the explanation for this lies along the line of work begun by Scholberg and Wallis (1910) who showed that there was an actual proteolysis taking place in milk with a high bacterial count during warm weather. This observation, combined with those of Savage (1925) and Arnold (1928, 5) can be interpreted as indicating that gastro-intestinal irritants are produced in the form of by-products of bacterial action upon certain proteins. This will be discussed further in connection with food-poisoning.

VI. EPIDEMIC WATER-BORNE DIARRHOEA.

Epidemics of diarrhoea have been observed to follow immediately after sudden pollution of a domestic water supply. The onset of these diarrhoea outbreaks is usually sudden. The clinical picture includes headache, nausea, pains in the abdomen and back, cramp in legs and diarrhoea. There is usually a sensation of chilliness or "shivers." Sometimes the diarrhoea and other symptoms only last one day, the longer in duration the greater the prostration. The temperature is seldom elevated more than one degree and subnormal temperatures are often observed. The tongue is furred-looking, the abdomen is tender and some tympanites is usually present. The outbreaks follow twenty-four to forty-eight hours after pollution of the water supply. Bacteriological examination of the excreta does not show the presence of pathogenic or foreign bacteria. The blood does not contain agglutinins against members of the typhoid-paratyphoid dysentery group after recovery. If a typhoid fever epidemic follows some two weeks after this outbreak of diarrhoea it is epidemiologically a water-borne typhoid epidemic. We have approached this problem from an experimental standpoint in the following manner:

Puppies were placed in warm and humid chambers and given sanitary and polluted water to drink. We will not deal with all the experimental work, but briefly cite two interesting experiments. The polluted water was obtained from the Chicago River close to the opening of a large sewer draining the congested business section (the so-called "loop" district of Chicago). The sanitary water was the chlorinated Lake Michigan water used for domestic purposes in Chicago. Twelve young dogs were placed in the warm room (90° F. temperature and 95 per cent. humidity), and given the polluted water; twelve young animals of similar size were given tap water in the same room. Each animal was given 100 c.c. of milk every four hours from 8 a.m. to 4 p.m. The experiment lasted three days. Two hours before killing the animals, in an ether chamber, they were given 100 c.c. of their respective water samples plus one agar slant of *B. prodigiosus*. Specimens were removed from the stomach, duodenum, upper jejunum, lower jejunum, ileum, and caecum, and the relative distribution of *B. prodigiosus* was determined in per cent. The same number of young dogs was used in an additional experiment in which we employed

sodium carbonate to bring the hydrogen-ion concentration of both drinking water samples up to *p*H 8·0. Otherwise the experiment was the same. Charts XVII–XVIII show the average results of these experiments. Eight

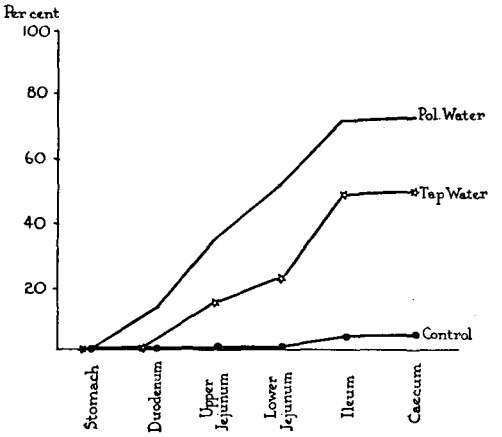

Chart XVII. Puppies fed on milk three times daily for three days. Two hours before they were killed each animal was given one agar slant of *B. prodigiosus* in water by mouth. Ordinate shows relative percentage of *B. prodigiosus*. Abscissa represents the various levels of the gastro-intestinal tract examined. Control shows relative distribution of *B. prodigiosus* throughout the intestinal tract when kept in ordinary temperature room and given tap water to drink. Tap water represents the relative distribution of *B. prodigiosus* throughout the alimentary canal when kept in hot room and given tap water to drink. Polluted water represents the relative distribution of *B. prodigiosus* when kept in hot room and given polluted water to drink.

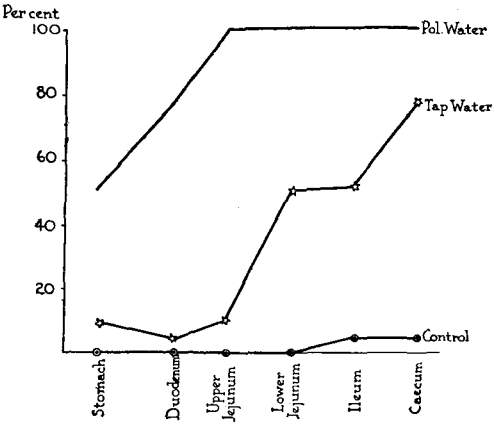

Chart XVIII. Puppies fed on milk three times daily for three days. Two hours before they were killed, each animal was given one agar slant of *B. prodigiosus* in water by mouth. Ordinate shows relative percentage of *B. prodigiosus*. Abscissa represents the various levels of the gastro-intestinal tract examined. Control shows relative concentration of *B. prodigiosus* throughout the gastro-intestinal tract when puppies are kept in ordinary temperature room and given alkaline tap water (*p*H 8·0). Tap water represents the relative concentration of the *B. prodigiosus* within the lumen of the digestive tube when puppies are kept in hot room and given alkaline tap water (*p*H 8·0). Polluted water represents the distribution of *B. prodigiosus* when puppies were confined to hot room and given alkaline polluted water to drink (*p*H 8·0).

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of the twelve alkaline polluted-water dogs had a diarrhoea on the second and the third day of the experiment. Six of the twelve alkaline tap-water dogs had loose green stools, but not a frank diarrhoea as was present in the other animals.

This experiment shows that the polluted water caused an inhibition of the self-disinfecting power of the alimentary canal, this action was accentuated by alkalinising the water. These experiments, with the preceding ones, show the changes that take place within the lumen of the small intestine in the experimental animal when there is an alteration in certain nutritional and climatic factors. The changes within the lumen of the gastro-intestinal tract leading to an inhibition of the normal bactericidal power is a part of the biological reaction of adaptation to environmental changes. We have found these changes to be as constant as changes in blood pressure, respiratory rate, expired carbon dioxide tension, acid-base equilibrium of the blood and urine and other physiological changes that have been studied during the process of adaptation to external stimuli of various kinds.

Bacteria ingested by such animals during diarrhoea remain in a viable condition throughout the whole length of the gastro-intestinal tract. Bacteria placed in the duodenum, as would take place in a chronic *B. typhosus* or *B. paratyphosus* carrier, would be excreted in the faeces in enormous numbers. It is conceivable that, under normal conditions, these bacteria entering the duodenum in the biliary secretions would be to a great extent destroyed before reaching the large intestine.

From our experimental work we are led to believe that there are several factors involved in determining the incidence of typhoid fever following such a water-borne epidemic of diarrhoea. In addition to the possibility of the polluted water containing *B. typhosus*, the gastro-intestinal irritating substances causing the diarrhoea increase the distribution of *B. typhosus* from carriers. These carriers may be harmless under ordinary conditions, the auto-disinfecting mechanism of the small intestine prevents viable *B. typhosus* from gaining entrance into the lower levels of the alimentary tract, but under the disturbed functional conditions associated with diarrhoea, these carriers become reservoirs of *B. typhosus*.

Juergens (1927) has raised some very interesting questions in relation to the 1926 typhoid epidemic in Hanover. There were estimated to be 35,000 to 40,000 cases of diarrhoea due to a pollution of the domestic water supply. Juergens shows that the typhoid fever epidemic that followed fourteen to twenty-eight days after this diarrhoea outbreak was not evenly distributed through the area supplied with the polluted water. In Linten-Nord the typhoid rate was 13·4 per 1000, as compared to a rate of from 2·7 to 5 per 1000 in other wards. The rate was highest in the unsanitary sections, where effective sewage disposal systems were absent, with crowded living conditions. Alstadt had a rate of 5·7 per 1000 and Neustadt 10·4 per 1000. Both of these sections have the same kind of population, but Neustadt is situated on low swampy

ground and has relatively poor sanitation; Altstadt lies on higher sandy ground and is more sanitary. This epidemiological report illustrates the importance of an unsanitary environment upon the development of enteric fever after water-borne outbreaks of diarrhoea. In the disturbance in the equilibrium between parasites and host, it is well to consider the increased distribution of *B. typhosus* from biliary carriers and the increased susceptibility of the population due to gastro-intestinal irritation.

VII. Enteric fever.

The seasonal distribution of enteric fever has long been known and several explanations have been offered regarding this phenomenon. The changes in the self-disinfecting power of the lumen of the gastro-intestinal tract in our experimental work has led us to believe that this reaction may play an important part in the series of reactions that take place between parasite and host, that lead to an increase in the incidence of enteric fever during the hot months of the year.

There is a loss of the power of the upper half of the small intestine to regulate and control its endogenous bacterial flora when there is an elevation in the external temperature and humidity. The bacteria fed by mouth tend to maintain themselves within the lumen of the intestinal tract. Bacteria placed in the duodenum of such animals are not destroyed in the small intestine in the usual manner, but appear in the large intestine within a short period of time and persist in a viable condition throughout the alimentary tract from the pylorus to the anus. This would increase the hazards of infection if pathogenic bacteria were ingested during hot weather. It is conceivable that biliary carriers of the gastro-intestinal infectious diseases are influenced by the same mechanism. If the bactericidal action of the small intestine is undisturbed, few of these bacteria should reach the large intestine. But on the other hand, if the elevation in external temperature causes a peripheral stimulation and reduces the activities of the splanchnic region, as was found in our experimental work, then the faeces of such biliary carriers should contain many of these pathogenic micro-organisms. Arnold (1927, 3) has discussed this problem in greater detail.

Cornwall and Le Frenais (1924) have shown that the stomach is not an efficient barrier against viable *B. typhosus* reaching the small intestine. Armstrong (1926) has analysed 449 milk-borne outbreaks of typhoid fever in the United States, 1908 to 1926, involving some 14,000 cases. The majority of these occurred during the hot months of the year. Freezer, Gibson and Mathews (1928) have shown that cows' milk is even better than the usual "alkalis" as therapeutic agents in controlling gastric acidity. It has long been known that it requires a large amount of acid to entirely neutralise the bound alkaline substances in cows' milk. Underhill and Simpson (1920) in the course of their study upon the excretion of indican and phenols found that a high protein diet in man during the summer season causes diarrhoea to such an extent

that the experiment had to be interrupted. Hoelzel (1926) has shown that a high protein diet tends to exhaust the power of the gastric mucosa to secrete hydrochloric acid in man. Diet, then, can play an important rôle in changing the susceptibility of the host to enteric diseases.

Semple and Greig (1908) in India, and Leach, Dehler and Havens (1926) in the southern part of the United States, report a carrier incidence of over 10 per cent. in recovered typhoid cases. This may be in a great part explained by the climatic conditions mentioned above. The possible increase in the number of carriers combined with diminution in the bactericidal action of the small intestine may play an important part in the seasonal distribution of enteric fevers. Kisskalt (1915) has assumed an increase in the number of carriers as an explanation for the summer seasonal incidence of such diseases. Knorr (1926) found, in a small group of paratyphoid carriers that were repeatedly examined, that there was an increase in the excretion of *B. paratyphosus* during the warm and hot months of the year.

Mills (1928) in China has called attention to certain gastro-intestinal disturbances, nausea, vomiting, epigastric pains followed by either diarrhoea or constipation, that are associated with sudden elevations in temperature and humidity. These cases usually show some hypochlorhydria and vasomotor hypotension. Mills states that foreigners are more susceptible than natives. These symptoms are associated with functional insufficiency of the suprarenal glands by this author. They can be explained upon the basis of changes within the body due to external stimuli as has been previously discussed in this paper. Mills administers epinephrine by mouth to these cases. The changes he describes are typical of peripheral irritation leading to a condition of parasympathetic status of the skin. The administration of epinephrine would tend to counterbalance this by a sympathetic stimulation. Many such illustrations could be cited from the literature. The biological functional balance between the peripheral and splanchnic systems of the body is of fundamental importance in the science of health and aids in understanding some of the symptomatology of disease.

VIII. CHOLERA.

Russell (1925–6) has recently published extensive studies upon the relationship between meteorological conditions and cholera epidemics in India. In order not to repeat what has been written in the previous discussion, the author wishes to outline in addition a few points that probably have a bearing upon this problem. Ross and Bagchi (1918–19) have shown that there is a seasonal variation in the acid-base content of the water of the Ganges. There is an unusually high alkaline content during the spring months. The close correlations Russell found between rainfall, humidity and temperature in the northern group, the one or two months' lag in the other two geographical groups used by him in this study, illustrate the variety of factors that must play a part. The lag of a month or two months in the peak of the cholera

epidemic behind the elevation in temperature and associated conditions could well be explained by changes in the host as well as by changes in the cholera micro-organism in the soil, etc. Cholera carriers may play a part. The better nourished population should react more slowly to the hot weather, the poorly nourished population should react with a shorter lag period. It seems more reasonable to assume a change in host susceptibility due to hot environment than to a Pettenkofer's "y" substance in the soil. If it is true, in addition to these factors analysed by Russell, that the population along the Ganges uses alkaline water part of the year and acid water at other seasons, this might play an important part.

The ingestion of dilute acids has long been a preventive agent against cholera. This subject has been dealt with by Arnold (1927, 4) in a previous communication.

IX. Food-poisoning.

Many outbreaks of "food-poisoning" are characterised by rapid onset and rapid recovery, also by the fact that no pathogenic bacteria can be isolated from the sufferers or from the food and by the fact that the sera of the patients do not as a rule show any development of agglutinins against the *paratyphosus* B. group of bacilli. A considerable number of these outbreaks are not reported to the health authorities, and of those reported a significant percentage are not recorded in the literature because of the negative findings. The mortality is low.

During the course of our study of the biological control of the endogenous bacterial flora of the gastro-intestinal tract, certain observations have been made that may throw some light upon the mechanism involved in host susceptibility to food-poisoning.

The *enteritidis* group used for this experimental study were kindly furnished by Prof. Jordan (J. 53, 179, 180, 206 and 222). Meat cultures were made from ground-up beef muscle with an equal amount of water and half of 1 per cent. sodium chloride. This was sterilised in the autoclave and inoculated with each of the five strains of *B. enteritidis*. The flasks were then incubated twenty-four hours at 37° C. and placed at room temperature until used. The flasks were placed in boiling water for one hour in the heated, infected meat experiments. Agar cultures of *B. prodigiosus* suspended in saline were added before feeding. These chromogenic bacteria have been found to be most satisfactory as test organisms for the intestinal bactericidal studies. Two c.c. of $N/1$ sodium hydroxide were added to each 100 c.c. of standard nutrient agar for detecting this test organism in the intestinal contents. This facilitates pigment production. Puppies, one to three months old, were fasted for twenty-four hours and fed with the test meal. The animals were placed in the hot room one to two hours before being fed. Two and a half hours after feeding, the puppies were anaesthetised and specimens removed from the stomach, duodenum, upper and lower jejunum, ileum and colon for bacterio-

logical examination. The contents of the stomach were removed and titrated for free and total acidity. Then the whole of the gastro-intestinal tract was examined for gross pathological lesions immediately after death of the animal. One hundred and fourteen puppies have so far been used in these experiments.

Table III shows the results of control experiments. The *B. enteritidis* cultures and vaccines did not exert an appreciable influence upon the bactericidal power of the intestinal tract. The differences between cool and hot room experiments are apparent.

Table III. *Relative distribution of* B. prodigiosus *through gastro-intestinal tract of puppies* $2\frac{1}{2}$ *hours after feeding with living and killed* B. enteritidis *cultures.*

No food	Before feeding	Stomach	Duodenum	Upper jejunum	Lower jejunum	Ileum	Caecum
Saline only.	%	%	%	%	%	%	%
Cool room*	100	0	0	0	0	10	10
B. enteritidis culture.							
Cool room	100	0	0	0	0	10	15
B. enteritidis vaccine.							
Cool room	100	0	0	0	0	10	20
Saline.							
Hot room†	100	25	0	0	0	25	35
B. enteritidis culture.							
Hot room	100	35	0	0	0	20	25
B. enteritidis vaccine.							
Hot room	100	25	0	0	0	25	25

* Ordinary room temperature.
† Temperature 95–98° F. Humidity 85–95 %.

Table IV shows that the heated *enteritidis* meat in the hot room causes an inhibition of the auto-disinfecting power of the gastro-intestinal tract. All of these animals are sluggish, most of them vomit and show some diarrhoea.

Table IV. *Distribution of* B. prodigiosus *through gastro-intestinal tract of three-months-old puppies* $2\frac{1}{2}$ *hours after feeding with plain and* enteritidis *infected meat in cool and hot rooms.*

Meat with bread	Before feeding	Stomach	Duodenum	Upper jejunum	Lower jejunum	Ileum	Caecum
Control plain meat.	%	%	%	%	%	%	%
Cool room*	100	0	0	0	0	0	10
Control plain meat.							
Hot room†	100	15	15	25	35	100	100
Enteritidis meat.							
Cool room	100	0	0	0	0	10	25
Enteritidis meat.							
Hot room	100	15	25	25	50	100	100
Heated *enteritidis* meat.							
Cool room	100	0	0	0	25	35	35
Heated *enteritidis* meat.							
Hot room	100	100	100	100	100	100	100

* Ordinary room temperature.
† Temperature 95–98° F. Humidity 85–95 %.

Table V shows that *enteritidis* infected milk does not contain the toxic substance that is present in the meat. This table also shows the beneficial

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effect of acidified milk upon the bactericidal power of the intestinal tract. The lactic acid was added just before feeding.

Table VI shows the results of gastric analysis two and a half hours after the various feedings. The depression of gastric acid secretion is apparent after feeding the heated *enteritidis* meat material.

B. coli does not produce toxic substances when grown in meat medium. *B. typhosus* causes some changes, occupying an intermediate position between the *B. enteritidis* and the control or *B. coli* meat.

Puppies, that have been injected with vaccines composed of the five strains of *paratyphosus-enteritidis* used in this work, are just as susceptible to the fed meat cultures of the same strains as the unvaccinated controls. If *enteritidis* infected meat is made alkaline (*p*H 8·0 to 8·5) with sodium hydroxide it produces more gastro-intestinal irritation, in fact such alkalinised unheated specimens can be made to produce diarrhoea in the majority of puppies in the hot room by this method. If such alkalinised Salmonella infected meat is heated, and fed to puppies in the hot room, most of them vomit within fifteen minutes and diarrhoea starts after an hour. These animals are very sick; about one-fourth of them die after two to four hours.

Schottmueller (1904) compared the acute gastro-enteritis due to the ingestion of certain members of the *B. paratyphosus* B. group to that caused by the chlorides of mercury. He considered the toxic agent an absorbable substance that acted upon the gastro-intestinal tract after absorption into the systemic circulation. Savage and White (1925) have shown that there are heat stable toxic substances in cultures of the Salmonella group that cause congestion and petechial haemorrhages in the stomach and duodenum when fed to kittens. We have substantiated this observation by using young dogs. The changes in gastric acidity and the bactericidal power of the intestinal tract are better indices than the appearance of gross pathological lesions in the mucosa of the stomach and duodenum. Jordan and Geiger (1923) have shown that inoculation with the usual triple vaccine fails to confer protection in an outbreak of food-poisoning due to the paratyphoid-enteritidis group. Ecker and Wolpaw (1926) substantiated this in an experimental study; these workers used rabbits. Savage and White came to the same conclusion in their work. We have substantiated these observations with the use of young dogs.

Certain strains of the *paratyphosus-enteritidis* group produce a toxic substance when grown in meat medium. These substances become more irritating when heated; this would lead one to think that they are probably metabolic products that increase upon hydrolysis. The gastro-intestinal tract of the grown dog is not appreciably affected by these substances. The puppy is sometimes susceptible, but when the puppy is placed in a warm chamber the gastro-intestinal tract is very susceptible to this irritant. There is a diminution in acid secretion, often-times an absence, with an actual increase in alkaline reacting material in the stomach. Vomiting and diarrhoea occur in

about half of these animals. Congestion and capillary haemorrhage is often-times present in the duodenum. There is a marked disturbance in the bacterial flora of the intestinal tract. *B. coli* appears in the duodenum and also in the stomach. The usual disinfecting power of the gastro-intestinal tract is interfered with and often-times entirely absent. Several strains of *B. coli* from the dogs, rabbits, guinea-pigs, rats, mice and man grown in meat medium do not produce toxic substances. *B. typhosus* occupies an intermediate position

Table V. *Relative distribution of* B. prodigiosus *through gastro-intestinal tract of puppies* $2\frac{1}{2}$ *hours after feeding with plain and* enteritidis *infected milk.*

Milk	Before feeding	Stomach	Duo-denum	Upper jejunum	Lower jejunum	Ileum	Caecum
Plain milk.	%	%	%	%	%	%	%
Hot room*	100	50	10	25	25	75	75
Enteritidis milk.							
Hot room	100	60	15	25	15	50	50
Heated *enteritidis* milk.							
Hot room	100	50	Trace	35	25	75	75
Lactic acid milk.							
Hot room	100	0	0	10	20	25	50
Acidified *enteritidis* milk.							
Hot room	100	0	0	0	15	15	50
Acidified heated *enteritidis* milk.							
Hot room	100	0	0	Trace	25	25	50

* Temperature 95–98° F. Humidity 85–95 %.

Table VI. *Acid-deficit in stomach of puppies* $2\frac{1}{2}$ *hours after feeding.*

Food	c.c. 0·1*N* HCl* per 100 c.c. food	Cool room c.c. 0·1*N* HCl* per 100 c.c. gastric contents	Hot room c.c. 0·1*N* HCl* per 100 c.c. gastric contents
Plain meat and bread	50	10	36
Enteritidis meat and bread	65	12	50
Heated *enteritidis* meat and bread	78	19	70

* Acid-deficit amount of acid necessary to bring content to *p*H 3·0.

between *B. coli* and the *B. enteritidis* in the production of this irritating material in meat medium. Milk cultures of the paratyphoid-enteritidis strains used in this work did not produce toxic substances. This latter observation may help to explain the relatively few outbreaks of food-poisoning due to milk. If the intestinal tract of the domestic animal is our reservoir for the *B. enteritidis* group, one would expect more milk than meat borne outbreaks due to the greater chance of contamination. Pasteurisation would tend to increase and not decrease the concentration of the irritating substances. Some of the body changes that followed the ingestion of a high meat meal have already been discussed. The interference with the usual self-disinfecting power of the small intestine accompanies the specific dynamic action that follows such a meal. Changes in the coagulability of the blood and certain alterations in the aqueous metabolism were mentioned. Attention was called to the resemblance of this reaction to a mild intoxication, such as follows the intradermal injection of a foreign protein, or possibly to certain climatic changes.

In this connection it may be significant that food-poisoning is usually due to meat and meat products. The biological changes that take place in the organism after the ingestion of meat may play a part in the susceptibility of the organism to the phenomenon of "food-poisoning." This question is being studied at the present time in our laboratory.

The food-poisoning outbreaks due to the Salmonella group show an increase during the hot summer months in England and Germany. This may well be due to a combination of three factors: an increase in the distribution of this group of bacteria due to the effect of external temperature upon the bactericidal power of the intestinal tract of domestic animals; an increase in the number of these bacteria in the medium; and lastly, a more susceptible gastro-intestinal tract of the human to these irritating substances.

X. Oral vaccination.

Many attempts have been made to immunise the host against certain diseases by oral administration of the antigen. Besredka (1927) and Calmette (1923) have recently reviewed the literature upon this subject. The problem of oral vaccination and local immunity are inseparable. Gay (1928) has clarified our views upon local immunity in several recent contributions. A thorough discussion of this question would be out of place in this brief summary of our experimental work. Wassermann and Citron (1911) suggested an increased sensitiveness or "retuning" ("Umstimmung") of certain areas of tissue that had come in contact with an antigen, and suggested that the increased resistance was due to this factor. They, as well as others (Hektoen, 1911), could not demonstrate anti-bodies in the blood serum in *in vitro* experiments. Besredka (1927) believes that the free phagocytes in their process of phagocytosis liberate from bacteria an "antivirus," which is essentially a non-antigenic toxic extract of the bacterial cells. This "antivirus" turns at once to the specialised receptive cells of the body which in turn are rapidly saturated with the "antivirus." This desensitises these cells from further toxic action of the homologous virus. The receptive cells in the enteric fevers are in the wall of the gastro-intestinal tract. Besredka administered bile in order to lower the natural resistance of the intestinal wall to the vaccine. "We can easily understand the mechanism of the decrease of immunity that follows, after the administration of bile. Bile being a powerful cholagogue, has a desquamating action on the superficial layer of the intestine. This layer being removed, the remains of the mucus are swept before it and so the intestinal wall becomes particularly permeable. A break is therefore made in the intestinal wall, and because of this, the paratyphoid infection becomes established" (Besredka, 1927, p. 120).

Arnold and Weiss (1926) used bacteriophage to dissolve bacteria and used these sterile filtrates of bacterial proteins as antigens in experimental animals. During the course of this work it was apparent that the protection enjoyed by the animal against a multiple lethal dose of the homologous bacteria was

out of proportion to the so-called "antibody" titre of the serum. This has been shown by numerous investigators using a variety of technical procedures.

Besredka, as mentioned above, used bile to "sensitise" the gastro-intestinal tract and facilitate the process of local immunisation after oral administration of an antigen. As a result of our experience with microbic intestinal permeability, we were convinced that the healthy intestinal wall could be so changed in its functional status as to allow bacteria to pass through it for a short period of time. These bacteria are found in greatest concentration in the splanchnic region, liver and mesenteric lymph nodes. If dogs are killed one-half hour after intra-duodenal injection of alkalinised bile, or alkalinised egg-white, or both of these, together with *B. prodigiosus*, *B. pyocyaneus*, *B. enteritidis* or *B. typhosus*, the respective bacteria can be demonstrated in the mesenteric lymph nodes and liver in large numbers. The lung shows the presence of the bacteria in culture in about half of these animals. The systemic blood (femoral artery or femoral vein) does not contain the test bacteria at any time when 5 c.c. of blood is added to 50 c.c. of broth at varying time intervals. One and a half hours after intra-duodenal injection, as mentioned above, the respective bacteria can be demonstrated in the liver and mesenteric lymph nodes by cultural methods. The lung does not contain viable test bacteria after this time interval. Three hours after such an injection, there are fewer bacteria in the liver and mesenteric lymph nodes, some specimens are negative. Eight hours after the same experiment, the injected bacteria cannot be found in the internal organs.

These experiments show that after a period of increased permeability of the intestinal tract most of the bacteria are in the splanchnic region; few enter the systemic blood stream via the thoracic duct and these are removed by the lung. This may play a part in pulmonary and mediastinal pathological findings in infants and even at times in adults. This of course is only suggested as a new angle of approach to an old problem.

We have already presented our evidence upon microbic permeability of the wall of the small intestine. The author wishes at this place to show the possibility of utilising this phenomenon to introduce antigenic substances into the body. Arnold and Finder (1928, 2) have shown that certain concentrations of bile placed in the dog's stomach leave this organ rapidly and do not stimulate acid secretion. Bile is an alkaline fluid. The duodenal reaction must be changed toward the alkaline side when this material is passed unchanged from the stomach into this organ. Another important factor is that in the fasting post-digestive state the administration of this alkaline bile is not followed by hydrochloric acid secretion. In other words, the splanchnic glandular system is not stimulated by certain concentrations of bile administered at a time when the stomach is empty. From our experimental work we are led to believe that in Besredka's method of oral vaccination the use of bile has another explanation than the one advanced by this investigator. We are now working upon this problem. The detailed reports will be published

at a future date. The increased resistance enjoyed as a result of oral vaccination in the experimental animals seems to reside in the splanchnic area, probably in the reticulo-endothelial apparatus in this region. Previous experience of this phagocytic mechanism with antigenic substances introduced during a period of disturbed autonomic equilibrium would not need new theories to account for the ability to efficiently phagocytise the same substance introduced at a later time. This may be locally within the wall of the intestine, but experiments indicate that the vascular and lymphatic splanchnic system, liver, etc., are all involved in this "retuning" or "Umstimmung" as expressed by Wassermann and Citron.

XI. Self-disinfecting power of the upper respiratory tract.

The author does not wish to fully discuss this problem here, but it is added to this discussion to show that the mucous membrane of this tract can regulate its endogenous bacterial flora as efficiently as the mucosa of the intestinal tract can control its own bacterial flora. Arnold, Ostrom and Singer (1928; 4) have shown that when bacteria are sprayed into the nose they are rendered non-viable within a short period of time. When several million *B. coli*, *B. prodigiosus*, or staphylococcus are sprayed into the nose most of them are destroyed within five minutes. The few remaining after ten to fifteen minutes have been grown in culture media and resprayed again. This second cultural growth is removed as efficiently as the first. This has been repeated as many as nine times. There was no evidence of an acquired resistance on the part of those bacteria that had survived for a maximum period of time upon the nasal mucosa. Bacteria were sprayed into the nose eight successive times at thirty minute intervals. There was no evidence of exhaustion of this disinfecting mechanism, in fact, the bacteria are removed at a more rapid rate upon successive applications. This is probably due in a great part to the mechanical irritation of repeated application of the swab to the mucous membrane. Nasal washings and also nasal secretions collected by packing the nose with dry sponges do not contain inhibiting substances for the same bacteria in *in vitro* experiments. Such secretions, collected after a period of repeated spraying of the nose with bacteria, do not contain inhibiting substances in various dilutions in broth cultures. In fact all of our results demonstrate growth-promoting substances in nasal secretions for the same bacteria that are rapidly rendered non-viable when placed upon the normal nasal mucosa.

Most experiments made upon human volunteers to transmit "common colds," "influenza" and other similar upper respiratory disturbances have been negative. Our experience with over six hundred experiments dealing with the power of the human nose to destroy exogenous bacteria has convinced us that this is a very efficient mechanism. As mentioned above, we only cite these experiments to emphasise that the mucous membrane of the upper respiratory tract has the power to destroy bacteria placed upon its surface.

XII. SUMMARY

There are certain demonstrable changes that take place within the gastro-intestinal tract during the process of adaptation to external stimuli. The body as a whole reacts in this adjustment to a changing environment. We have presented our evidence and indicated some instances where these gastro-intestinal changes can be considered significant from an epidemiological and preventive medicine standpoint.

The loss of the self-disinfecting power, changes in acid-base equilibrium, and other demonstrable alterations within the lumen of the alimentary tract are only a part of the changes taking place in the organism during the process of adaptation to external environmental changes. If certain diseases are associated with a disturbance of the equilibrium between the parasites and the host, then it naturally follows that we must have more information upon the biological mechanism of the host in regard to its changes in susceptibility and resistance to the parasitic environment. Our present knowledge of bacteriology throws little light upon this epidemiological question. It has been the aim of the author to study the mechanism by which the organism adapts itself to certain environmental changes. We have avoided using pharmacological and other artificial agents that would produce body changes, but have restricted ourselves to simple alterations in diet, external temperature and similar mild or natural environmental factors, and followed the mechanism of adaptation to these physiological stimuli.

The loss of the power of a body-covering layer, to destroy bacteria in contact with it, must increase the hazards of invasion of this body surface by bacteria. We have shown that a sudden change in the intra-intestinal acid-base equilibrium leads to an inhibition of the mucosa to destroy bacteria within its lumen and is accompanied at times by the appearance of viable bacteria in the thoracic duct lymph stream.

If an organism does not adapt itself to its meteorological environment, it is more susceptible to its parasitic environment. The same is true of the nutritional environmental factors. Disturbances due to lack of adaptation to climatic changes can in part be offset by altering the diet. The weather and food factors cannot be separated and one can influence the other.

Changes in climate are beneficial to the well-being of the population living in the temperate zones where meteorological conditions are ever changing as compared to the steady cold in the arctic or constant heat as in the tropics. Changes in food are also beneficial. A high protein meal is stimulating. In the tropics where the skin is in a state of over-stimulation due to the high external temperature and humidity the ingestion of an alcoholic beverage to stimulate the splanchnic system before a meal is beneficial. Acidifying an infant's food in hot weather is a common practice. Many such instances could be cited. It seems to be of advantage to an organism to be in a changing environment. The tonus of our physiological systems is not static or fixed, but is ever

changing. The more delicate the index used to measure functional changes, the more frequently are such changes observed. These alterations in environment must not exceed the power of the organism to adapt itself to these changes.

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