# ROENTGEN RAY INTOXICATION.

## I. Bacterial Invasion of the Blood Stream as Influenced by X-Ray Destruction of the Mucosal Epithelium of the Small Intestine.

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It is a truism accepted by physiologists, bacteriologists, and pathologists alike that the covering mucosal epithelium of the intestinal tract is a very important if not the all important barrier which guards the underlying tissues, lymph, and blood vessels from overwhelming invasion by hoards of bacteria which swarm in the intestinal tract. Our experiments indicate strongly that this barrier may be wiped away completely and yet the expected overwhelming invasion does not follow. Evidently the other tissues or tissue juices have a protective mechanism, perhaps a form of secondary defense which is of much importance and is not to be neglected in estimating the resistance of the intestine to bacterial invasion. If this observation is correct this phenomenon is worthy of much careful study and should interest a variety of investigators, particularly the bacteriologist and the pathologist.

Warren and Whipple (58) have described in sufficient detail the remarkable influence of large doses of the x-ray upon the epithelium of the small intestine. Suitable doses of x-rays in normal dogs will destroy almost completely the epithelial covering of the villi and crypts of the small intestine. Such experiments present a remarkable picture in which the intestinal lumen is devoid of its epithelial lining and the naked villi and denuded crypts are covered by masses of exudate and bacteria. It is difficult to believe that under such conditions the

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animal is not overwhelmed by bacterial invasion but the expected result does not follow and the inflammatory reaction is surprisingly slight.

Such experimental animals die usually on the 4th day with all clinical evidence of acute intoxication which in many respects is like that observed in intestinal obstruction. There is evidence that this x-ray intoxication is related to the epithelial cell disintegration in the mucosa but more experiments are needed to establish this point. We have observed toxic substances of proteose-like nature obtained from these injured intestines but wish to reserve a discussion of this subject for a subsequent publication.

These experiments are a continuation of the investigative program begun by Hall and Whipple (21) and extended by Warren and Whipple (58). This paper must be looked upon as a preliminary communication and the writers emphasize its incompleteness, but circumstances made it impossible for them to continue this investigation to furnish the needful quantitative measurement of bacterial invasion.

A considerable literature has accumulated in the past few years dealing with the permeability of the intestinal mucosa of various animals. The permeability of the normal and the injured mucosa has been investigated and much has been written concerning agonal and postmortem invasion of the blood stream from the intestinal tract. If space permitted it would be desirable to review some of these papers but we shall list merely the papers which have points of interest in relation to this problem.

There has been much discussion about the permeability of the normal *uninjured intestinal mucosa* to bacteria residing in the intestinal canal. Different investigators have obtained varying results with supposedly normal laboratory animals. Interesting data are to be found in papers by Trombetta (56), Metchnikoff (37), Korkunoff (32), Neisser (40), Opitz (44), Klimenko (31), Selter (46), Hornemann (28), Nocard (42), Nicolas and Descos (41), Adami (2), Ficker (15), Wrzosek (63), Holle (26), Tsunoda (57), von Wassermann and Sommerfeld (59), TenBroeck (54, 55), and Besredka (7).

The part played by intestinal injury or abnormality was investigated early in the study of invasion of the intestinal tract by bacteria. Papers dealing with intestinal incarceration, mechanical and chemical injury, parasitic injury of the intestine, and acute inflammation are reported by Oker-Blom (43), Bosc and Blanc (10), Neisser (40), Beco (5, 6), Flexner (16, 17), Birch-Hirschfeld (8), Casagrandi

(13), Arnd (3), Bosc (9), Sordoillet (49), Maklezow (35), Marfan and Bernard (36), Opitz (44), Klimenko (31), Weinberg (60), Southard and Canavan (50), C. Hess (22), O. Hess (23), Shearman and Moorhead (47), Hirst (24), and Fredette (18).

Agonal and postmortem invasion from the intestine has been carefully studied and much interesting data are to be found in papers by Trombetta (56), Beco (5, 6), Opitz (44), Klimenko (31), von Wassermann and Sommerfeld (59), Gwyn and Harris (20), Fredette (18), Southard and Canavan (12, 50), Richey and Goehring (45), Hiss and Zinsser (25), Achard and Phulpin (1), and Gay and Southard (19).

If bacteria reach the circulation their distribution in the body conforms to fairly well established mechanisms. These points are covered in papers by Ito (29), Meyer (38), Bartlett and Ozaki (4), Wyssokowitsch (64), O. Hess (23), Werigo (61, 62), Kyes (34), Bull (11), Hopkins and Parker (27), TenBroeck (54, 55), Teale and Bach (52, 53), Siebel (48), and Drinker and Shaw (14).

*Latent Organisms.*—In cultural studies in animals following a profound injury, there is another source for bacteria besides immediate invasion from the intestinal tract. Koser and McClelland (33) and later Teale and Bach (52, 53) pointed out that spores could be identified in the organs of animals days and weeks after injection. Teale and Bach showed further that if an animal in this state of equilibrium received an injection of almost any non-specific substance which gave a shock to the animal organism, the equilibrium would be upset often with fatal result. Perhaps some of our cultures of sporulating organisms can be explained on this basis.

*Diarrhea.*—Still another factor of importance which may influence bacterial invasion is the rapid and continuous emptying of the intestinal canal. Kendall (30) thinks that cathartics decrease the number of bacteria in the intestine by mechanical means. He offers no experimental data but cites Morax (39), Steiff (51), and others who studied the disinfectant action of various drugs in intestinal putrefaction.

## Method.

The dogs used in these experiments were normal and healthy animals. They were killed under chloroform anesthesia by bleeding. The body was washed with lysol and immediately opened with sterile precautions. Small blocks of tissue were taken from the lungs, liver, kidney, and mesenteric lymph nodes. Unfortunately the spleen was not cultured nor were quantitative tests made. These blocks were seared and small bits from the inside were cultured in 1 per cent glucose broth for 24 hours. 25 cc. of heart's blood were put into 250 cc. of 1 per cent glucose and also cultured for 24 hours. Enrichment cultures were plated on Endo's and plain agar medium. As a rule enrichments were inoculated in beef heart media aerobically and anaerobically. *Bacillus coli* was identified by the usual sugar tests.

Smears of the intestinal contents and of the organs were made at the time of autopsy. Smears of the organs were usually negative though an occasional Gram-positive rod (*sporogenes* group) was seen. The intestinal contents showed the usual Gram-negative rods (*Bacillus coli*), enterococci, and small Gram-positive cocci and Gram-positive rods, mostly of the *sporogenes* group, though the fermentative *Bacillus welchii* was frequently found. A number of sections from the intestines, liver, and kidney of normal dogs and the radiated animals were stained with a modified Gram stain and counterstained with methylene blue for the purpose of locating and differentiating some of the organisms in these tissues. Duplicates were stained with carbolthionine. It is evident that the search for bacteria in tissues except in the case of local invasion in large numbers is apt to be futile.

### EXPERIMENTAL OBSERVATIONS.

Table I represents the results of the cultural studies. *Bacillus sporogenes* was identified in the organs of the normal dog. Cocci and other organisms may also be cultured under apparently normal conditions from the mesenteric lymph nodes. Dog 19-130, sacrificed 24 hours after radiation over the abdomen, showed no qualitative evidence of invasion by other organisms. There was histological evidence of injury to the chromatin of the crypt epithelium of the small intestine[1] but there was no cell disintegration nor were there symptoms of intoxication at this time. This animal, therefore, may be looked upon as a second control. *Bacillus coli* was not found in either of these two dogs. It was not usually present except under abnormal conditions (*vide infra*).

Dog 19-101 was sacrificed 48 hours after radiation. This is the earliest period at which the first symptoms of clinical intoxication were manifest. Histologically many of the cells of the crypt epithelium were disintegrated and had fallen away from their basement membranes over a large part of the small intestine. *Bacillus coli* was cultured from the mesenteric lymph nodes, the left lung, and the right kidney. This would seem to indicate the invasion of the lymph stream, though vomiting and inspiration of this matter into

[1] Warren and Whipple, Paper II, this series.

TABLE I.

*Culture of Organs of Normal and Radiated Animals.*

| Organs. | Dog No. | | | | | |
|---|---|---|---|---|---|---|
| | 19-133 | 19-130 | 19-101 | 19-50 | 19-85 | 19-78 |
| | Normal control. | 24 hrs. after radiation. | 48 hrs. after radiation. | 72 hrs. after radiation. | 96 hrs. after radiation. | 96 hrs. after radiation (moribund). |
| Heart's blood. | Sterile. | Sterile. | Sterile. | Sterile. | One or two B. sporogenes. | B. coli; B. sporogenes. |
| Mesenteric lymph node. | Staphylococcus. | Staphylococcus. | B. coli; Staphylococcus albus. | B. coli; staphylococci; B. sporogenes. | B. coli; B. welchii; B. sporogenes. | B. coli; B. sporogenes. |
| Right lung. | A few B. sporogenes. | — | B. sporogenes. | B. sporogenes. | B. welchii; B. sporogenes. | B. coli; enterococci; B. sporogenes. |
| Left " | A few B. sporogenes. | " | " coli; Staphylococcus aureus. | " | B. sporogenes. | B. coli; cocci; B. sporogenes. |
| Right lobe of liver. | B. sporogenes. | " | B. sporogenes. | " | " welchii; B. sporogenes. | B. sporogenes. |
| Left " " " | " | " | " | " | B. sporogenes. | B. coli; B. sporogenes. |
| Right kidney. | " | Sterile. | " coli; Staphylococcus albus. | Sterile. | " coli; B. welchii; B. sporogenes. | Staphylococcus albus. |
| Left " | " | " | B. sporogenes. | " | B. welchii; B. sporogenes. | Sterile. |

the lung may be responsible for the positive culture of *Bacillus coli* from the left lung. The leucocytes which were infiltrating the submucosa were not present in great numbers. They seemed concerned with the remarkable injury to the crypt epithelium rather than with bacterial invasion.

Dog 19–50 sacrificed 72 hours after radiation was much intoxicated and showed slightly greater destruction of crypt cells than Dog 19–101. The invasion of *Bacillus coli* was found only in the mesenteric lymph nodes. Otherwise the type of organisms appeared to conform to those normally present. The usual invaders may or may not have been increased in numbers but many quantitative cultures must be made to settle this point.

Of the two dogs sacrificed 96 hours after radiation, Dog 19-85 was acutely intoxicated although not yet moribund. Dog 19-78 was moribund. The mucosa of the intestine was practically destroyed in both dogs. For the first time (Dog 19-85) the heart's blood was not sterile. *Bacillus sporogenes* was found in all the organs. *Bacillus coli* was found in the mesenteric lymph nodes and the right kidney. *Bacillus welchii* was also found there and in the right lobe of the liver, the right lung, and the left kidney.

In Dog 19-78, which was sacrificed while moribund, *Bacillus coli* was found in the heart's blood as well as *Bacillus sporogenes*. *Bacillus coli* was also present in the mesenteric lymph nodes, both lungs, and the left lobe of the liver. Cocci were found in the lungs and right kidney. It is rather remarkable that the left kidney should remain sterile in the moribund dog though in the 48 hour dog and the other 96 hour dog none of the organs were sterile.

Study of the *stained slides* from the *fixed sections* showed large masses of bacteria, mostly Gram-positive rods, in the lumen of the ileum and colon and between the villi and in the crypts. Very few bacteria were seen in the duodenum. In the markedly injured sections bacteria were found among the disintegrated epithelial cells and up against the basement membrane where the epithelial cells have fallen away. At no place were any organisms found to have invaded the stroma even in the sections where the villi had collapsed and open capillaries were present. Bacteria were not observed in the few leucocytes present. Kidneys and liver when culturally positive

showed no bacteria even after prolonged search. As pointed out above, this is to be expected as only a few organisms may be present and these may be missed or they may have been washed out by the histological method.

There was no evidence of local bacterial invasion of the intestinal wall at any point. It is to be noted that *Ascaris* and tapeworms were present in all these dogs.

### DISCUSSION.

It is now pretty generally accepted that the *normal mucosa* of the small intestine of the dog is actually permeable at times to the normal bacterial flora. The critic will insist that such invasion does in effect give good evidence for actual injury of the mucosa by food particles (bones, etc.). He will stress the importance of the frequent intestinal parasites of the common laboratory animals. He may further argue that much of the evidence for permeability of the normal mucosa is obtained by the use of bacteria foreign to the intestinal tract and this very procedure introduces an abnormal factor.

*Actual injury* of the intestinal wall gives an obvious means of bacterial invasion from the intestinal lumen. Invagination and strangulation of the gut will give not only cultural evidence of bacterial invasion within 6 to 15 hours but histological evidence of overwhelming invasion of local tissues. The natural deduction is that small injuries will likewise permit a bacterial invasion from the intestinal lumen, for example, enteritis, purgation, intestinal parasites. But we must be careful when we talk about constipation and simple catharsis and round worms as all opening the portals to bacterial invasion. This fact has not been established by any experimental data known to us and much careful *quantitative* cultural work with carefully controlled and autopsied animals will be required for the solution of this important problem. The *normal* has never been established beyond question and the very term often requires definition in various papers.

*Agonal* invasion of the blood stream and organs is not rare—in fact is quite common in all conditions but especially when there is abnormality of the intestinal tract. The importance of bacterial cultures in the agonal period must then be interpreted with a proper

understanding of this fact. Multiplication of organisms in the *post-mortem period* will furthur complicate a complex picture. Such postmortem observations are of limited value in a study of this type.

The heart's blood cultures in our experiments remained sterile until the last day of intoxication or until almost the agonal period. The same observation might hold for fatal respiratory distemper. It is common knowledge that many bacteria may be introduced into the blood stream without causing a bacteremia in dogs. The lungs, liver, and spleen are the important filters.

In some of the experiments of Hall and Whipple (21) the dogs survived the 4th day of intoxication and died 3 to 6 days later. Some of these dogs showed miliary kidney abscesses, liver focal necrosis, tiny infarcts in organs, and patches of pneumonia. It might be claimed that this is a result of bacterial invasion from the intestinal tract and in fact this may be the truth. But there is ground for argument to the effect that this is an invasion from the respiratory tract due to the severe intoxication, constant vomiting, and low resistance. We have found no similar lesions in this more recent series of dogs. Also a considerable series of rats and other animals exposed to x-rays failed to show abscesses or focal necroses in organs at autopsy.

We have a striking contrast in the intestinal mucosa between *excretion* or elimination or extravasation of fluid and the *absorption* following x-ray exposure. There is a *profuse excretion* throughout the intestinal tract and clinically the result is vomiting and diarrhea in spite of lack of food or fluid intake. We note escape of fluid through the mucosa, outwandering of white cells, and diapedesis, or hemorrhage of red cells. There is obvious *lack of absorption* and we note a surprising lack of bacterial invasion of the submucosa in spite of the total absence of the covering epithelium of crypts and villi. This preponderance of *flow out* through the mucosa may in fact be one of the protective factors which stand between the bacteria of the intestine and the tissues.

The mesenteric glands show evidence of a little invasion from the intestine during the 2nd, 3rd, and 4th days after x-ray exposure. It is probable that a mild enteritis would give a similar picture but here abnormalities of the mucosa would be hard to demonstrate histologically.

The outstanding feature of these experiments is the striking contrast between obvious injury of the intestinal epithelium and the lack of invasion of the intestinal bacteria into the tissues, lymph, and blood. We recall from the histology of this condition that during the 2nd and 3rd days after radiation the intestinal epithelium shows profound injury, disintegration, and separation from the basement membrane. Organ cultures at this time show almost the same picture as in control periods (Table I). During the 4th day after radiation there is severe clinical intoxication and often a preagonal coma. Organ cultures at this time show evidences of invasion of blood stream and considerable bacterial dissemination in various organs. But we might observe the same sequence in any severe intoxication leading to coma and death with complete absence of gross intestinal abnormality.

## SUMMARY.

The x-ray has a specific effect upon the epithelium lining the crypts and covering the villi of the small intestine. A suitable dose of x-ray will destroy this epithelium in large measure, leaving empty crypts and naked villi exposed to swarms of bacteria in the intestine. Subsequently one does not observe an overwhelming invasion of the tissues, lymph, and blood by intestinal bacteria. It seems obvious therefore that the intestinal epithelium is not the all important barrier which protects the tissues from invasion by intestinal bacteria.

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