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Experimental roentgenotherapy in peritonitis

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EXPERIMENTAL ROENTGENOTHERAPY IN PERITONITIS

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INDEX

I. INTRODUCTION

INTRODUCTION

When Roentgen discovered the X-rays in 1895 little did he realize the extent to which these rays would be used. They are now incorporated in every phase of science, and research is constantly being carried on to increase their application. In the field of medicine they serve as both therapeutic and diagnostic agents.

It is the purpose of this paper to discuss experiments with X-ray therapy and specifically peritonitis with its complications.

II. DEFINITIONS AND DESCRIPTIONS

DEFINITIONS AND DESCRIPTIONS

There are three terms which should be clarified as to their meaning and importance. They are Roentgenotherapy, Peritoneum and Peritonitis.

According to Stedman (1), roentgenotherapy is, "The treatment of diseases by means of the Roentgen rays".

One of the best descriptions of the peritoneum is given by Hertzler (2). He states, "The peritoneum is a serous membrane which covers the viscera and lines the walls of the abdominal cavity. It is coextensive with the cutaneous surface of the body, each comprising about 25,000 square inches. It is composed of a covering layer of flat, serrated cells, variously called endothelial or mesothelial, beneath which is a basement layer of connective tissue. With the peritoneum must be reckoned also the blood vessels and lymphatics which lie beneath the basement membrane, which give to it many of its functions and fix its importance in pathology and clinical surgery. The two chief functions of the peritoneum are (a) its power of absorption and exudation, by virtue of which the visceral coats are maintained in a condition which permits them to glide over one another without friction, and (b) its ability to form adhesions when injured, either mechanically or by infections."

Christopher (3) states that peritonitis is an infection of the peritoneum. There are two main types, aseptic and septic. Aseptic is caused by any irritant other than bacteria. It depends entirely upon the amount of the agent, the extent and the organs involved. For example, there can be a tubal abortion with the escape of blood into the peritoneal cavity. Bile is another agent, however, there is usually some other infection of a bacterial nature involved because any factor which permits the escape of bile will usually permit the escape of bacteria.

Septic peritonitis means a definite invasion of the peritoneum by bacteria. The infection may be caused by a single organism, but more often by a mixture of bacteria. The more common ones are B. Coli, B. pyocyaneus and streptococcus.

Hertzler (4) classifies septic peritonitis into three groups; first, localized peritonitis, second, spreading peritonitis, third, diffuse peritonitis, The localized, as the name implies, including those cases of peritonitis in which the inflammation has not spread beyond the primary point of infection. The term means that it is usually so walled in by adhesions that it is rela-

tively harmless for the time being.

Spreading peritonitis is exactly as the name implies. It is bound in only by the walls of the local organs and the adhesions between them. The appendix is the most common offender. The end result is that either the process becomes localized or the patient dies.

Diffuse peritonitis consists of rapidly spreading bacterial process as a result of a sudden pouring into the peritoneal cavity of a large amount of infectious material. There has been no preparatory reaction either due to the sudden onset or a lack of proper body defenses. Peritonitis is still a very important disease in spite of recent chemotherapy. Sulfonamides have markedly reduced the death rate in this disease and show definite promise of even greater advances. Nevertheless, patients still have peritonitis and some of them still die from it. Definite figures vary in different localities and in different hands.

Harvey (5) states in an article published in February of 1942 that the need for new means of preventing postoperative peritonitis in abdominal surgery is still great in spite of advances in surgical technique and pre- and postoperative care during the past decade.

At the Presbyterian Hospital in the period 1933 to 1939 inclusive, 316 radical resections were done for carcinoma of the rectum and colon. The post-operative mortality in five of these years lay between 13 and 19 percent, but two bad years raised the average to 20.3 percent. This represents the work of the staff as a whole. One member, Charles L. Janssen, who did nearly one-half of the abdominoperineal resections for carcinoma of the rectum, had a mortality rate which was about one-third that of the staff as a whole, and in the last three years of his life performed 27 operations with but 1 death. But the records of a few specially trained and gifted operators do not represent the difficulty experienced by the surgical profession the country over in preventing postoperative deaths after major operations on the intestinal tract.

It is imperative that we continue our search for therapeutic improvement, especially from a prophylactic standpoint. It is with this in mind that we investigate the possibilities of roentgenotherapy.

III. HISTORY

HISTORY

In order to become established, any means of therapy in any field must first go through the experimental and research stage.before it can be given any actual application as a therapeutic agent. The medical profession have a high regard for the individual. For that reason all early research and experimentation are done on animals rather than man. This has its disadvantages in that first there are inumerable types and species of animals used, and second, it is not always possible to transpose the results of the animals upon the human. Nevertheless, it is interesting to review briefly the animal experiments of peritonitis in general which have gone before, in an attempt to find and evaluate the most effective means of therapy.

Issaef (6) showed that intraperitoneal injection of many foreign substances in a guinea pig gave them an immunity to cholera organisms which were injected several days later. The substances experimented with included serum, broth, urine, and a 2 per cent nucleic acid solution. The degree of protection was at no time more than 15 times the amount which would produce death in a normal animal.

Harvey (7) in his survey of the history of peritonitis states that Mikuliez in 1904 reported Miyake's experiments in which intraperitoneal and subcutaneous injections of various substances were shown to give protection against organisms of the colon group or intestinal contents introduced intraperitoneally afterwards. Von Mikulicz had used nucleic acid, neutralized in 2 percent strength subcutaneously in fifty-five human beings before abdominal operations, and was pleased with the postoperative course. He explained the beneficial effect by the leucocytosis which the nucleic acid called forth. He tried one intraperitoneal injection of this substance, but it caused so much distress that he did not attempt this route again.

Herrmann (8) in 1928 was able to produce a certain amount of immunity by means of a mixed streptococcus and colon vaccine given intraperitoneally.

In 1932 Steinberg and Goldblatt (9) published their experimental work on dogs. They produced a bacteriogen composed of gum tragacanth, aleuronat, and the vaccine of a special strain of E. Coli. This was given intraperitoneally and produced a sterile peritonitis. They reported a definite peritoneal immunity.

Seley (10) in 1939 produced a suppurative peritonitis in cats by suspending a human strain of B. Coli in gastric mucin. He used no therapy but demonstrated that an equal number of B. Coli were not lethal unless suspended in gastric mucin.

Harvey (11) and his coworkers attempted to measure the comparative value of different types of therapy in peritonitis produced in animals, by intraperitoneal injection of living organisms. They used mice and guinea pigs for this part of the experiment. They used five methods:

1. Protection by means of living organisms.
2. Protection by filtrates.
3. Sera from rabbits and horses previously innoculated with E. Coli.
4. Vaccines prepared from intestinal organisms.
5. A variety of substances including typhoid vaccine, broth, saline solution, acetic acid, starch mixtures and sulfanilamide.

As a result he states: "In general, the degree of immunity we obtained was highest when the peritoneal reaction was most intense. In fact, animals that were made so sick by the preliminary injection that they appeared about to die often withstood the largest doses of infecting organisms. Such intense peritoneal reaction could be produced by nonspecific substances like aleuronat and starch mixture, or by bacterial products like E. Coli vaccine, and the protection afforded by each was in general the same.

"We therefore look on this protection as a function of the intensity of the peritoneal reaction, short of actually causing the death of the animal, and also as a nonspecific phenomenon, whose efficacy against any infecting organisms is independent of the substance used to produce it. An exception to this statement is the protection afforded by E. Coli, bacteriophage, which was effective against its own homologous strain of E. Coli, but ineffective against other strains of coli or against other organisms. The protection obtained by drugs may also be of a different nature."

He drew the following conclusions from the mice and guinea pig experiment:

1. Any substance injected into the peritoneal cavity of mice or guinea pigs produces a peritoneal reaction.
2. If the reaction is severe enough, it is accompanied by a measurable degree of immunity against subsequent intraperitoneal injection of various organisms.
3. The immunity appears to be nonspecific, except in the case of certain substances like bacteriophage in which the protection afforded appears to be due chiefly to its specific bacteridical property.
4. The degree of immunity obtained may reach several thousand minimal lethal doses against relatively virulent organisms, such as strains of E. Coli whose virulence has been artificially raised. E. Coli, enterococci, Cl. welchii, and other intestinal organisms, as obtained from the intestinal tract or from pus, are in our experiences always of low virulence, and against these strains the degree of immunity is usually of the order of ten minimal lethal doses. This is true also of intestinal contents, filtered through cotton.

5. He found no single protecting agent that was of outstanding value.

Harvey later used dogs. The peritonitis was produced by ligating the appendix and the blood supply about 4 cm from the tip. The appendix was then opened and the fecal contents removed, after which the appendix was placed back into the peritoneal cavity. A T tube was then placed into the midportion of the small intestine for enteral administration of drugs, and brought out through a close-fitting separate tract. The results are briefly:

Agent Used.	Total No. Dogs.	Per Cent Survived
Control	16	
Bacteragen	10	
Sulfathiazole (enterally)	12	
Sulfathiazole (intraperitoneal)	6	
Sulfa pyridine	8	
Sulfanilamide	4	
Sulfanilamide (intraperitoneally)	8	
E. Coli vaccine	8	
Bacteriophage	2	

Mischtschenko (12) in 1935 reviewed the situation at that time in an article entitles "Experimental foundations of roentgen therapy in acute inflammatory disease". His conclusions bear repeating at this time. They are:

1. "In roentgen therapy of acute inflammatory processes, several factors are of importance; the quality of radiant energy, the correct dosage, and time of irradia-

tion. Optimum doses for inflammatory foci are 80 per cent s.e.d. for rabbits, 60 per cent for guinea pigs, 20-30 per cent for humans. The beginning stage requires higher dosages, whereas the healing stage requires weak dosages, intensive radiation during the regenerative stage being only detrimental.

2. Morphologic examination of the blood and the local cell reactions in the tissues of acute inflammation show that irradiation produces an increase in destruction of leukocytes, an increase of phagocytosis and of the histiocytic reaction. These correspond to the clinical course whereby the inflammatory focus becomes smaller and the recovery period is considerably shortened. Very high doses cause a break in the defense barriers, a further spread of the inflammation, the development of phlegmons and finally generalized spread and sepsis.

3. Irradiation calls forth nonspecific antibodies in the blood.

4. Roentgen rays influence colloids so that there is a lowering of osmotic pressure, a lessening of exudation and wandering cells, and a conversion of surface tension.

5. Amino acids in inflammatory foci are increased by irradiation and, parallel with this, there is an increase

in trypsin. In irradiated inflammatory areas, there is an increased content of total protein, especially of its globulin fraction. The variations depend upon the degree of hyperemia in the tissues. Pepsin is not demonstrable.

6. Investigation of the vascular reaction of inflammed tissues (rabbits' ears) under the influence of varying doses of roentgen rays given for fifteen to sixty minutes showed no evidence of direct irradiation effect on the blood vessels.

7. The pain-relieving action of roentgen rays in inflammation is due to the influence of stored protein-split products and lipoids on the nerve endings, by lessening the tension and edema, transforming the tissue juices obtained from the colloids and solution of the crystalloids. Irradiation causes, therefore, an interruption in the pathways of the pain reflex."

Manges and Smith (13) in a review of the literature up to 1935 on experimental roentgentherapy stated that most of the work up to that time had been done on small animals such as mice, guinea pigs and rabbits. Dogs were seldom used. In most cases the amount of radiation was extremely high in comparison to doses given in actual treatment of infections of inflammations, so that some of the results

must be necessarily questioned.

"Many of the authors approached the subject by way of a study of the radiation effect on the blood, the blood forming organs, and antibodies produced by the blood, and the effect of radiation on specific immune bodies. There is rather definite agreement that the lymphocytes and reticuloendothelial cells are most easily destroyed by radiation and that the polymorphonuclear cells are most resistant to radiation, and naturally radiation even in heavy dosage over small areas of non-blood forming organs has little effect whereas total irradiation or irradiation over the important blood forming organs has marked effect. There is, too, pretty general agreement that irradiation has little or no effect on antibody fo tion in any but massive doses if radiation is given after t e process of formation is established."

They drew the conclusion hat at that time, clinical data was of far greater importa ce than experimental data. Probably because there was s h a variation in the amount of x-ray given, the type and size of animals and the uncertainty of some of the results.

The reaction which occurs when the body cell is subjected to x-ray is still a mystery. We do know different cells react differently, and that the same cell under different circumstances will show a variety of results. Diseased tissue and neoplasms have a different response compared to normal tissue. This reaction may be altered by diff rent physical agents and the condition of the body locally and generally.

In an attempt to straighten out the conflicting of the previous experiments and statements it is ry to attack the problem from a more fundamental basis. Namely, what is the effect of x-ray upon the individual cell, tissue and organs? How do different amounts of x-r y effect the body tissue? How long after exposure to x- y does the tissue show the greatest resistance? If we can answer questions by means of experiments thán we are closer to the actual solution and evaluation of x-ray therapy in inf ctious diseases in general and peritonitis especially. As pre iously stated we will not involve any experiments or discus ions about neoplasms.

In 1926 Ewing (14) felt that x-ray therapy was ef-

fective only in the total amount given. He stated that immediately following a treatment there was a change in the tissues which as yet could not be definitely described but it appeared to be generally an engorgment which was greatest from about 48 hours to 72 hours after the treatment.

Lewis (15) in 1927 compared the local effect of histamine and x-ray upon the skin. He described the histamine reaction as a "triple response".

The first component of this reaction is a local dilatation of the "minute vessels" of the skin, resulting in a red spot at the site of the histamine injection. The second phase is a widespread dilation of the neighboring "strong arterioles" causing a bright scarlet red halo known as the flare. The third part of the triple response consists of a local increase of permeability of the walls of the minute vessels. This results in the formation of a wheal at the site of the red spot described above. The first and third parts of the triple response are due to the direct action of histamine on the walls of the "minute vessels". The second part (i.e. the flare) results from the activation of the local axon reflex by the histamine which in turn causes an active arteriolar dilatation. Flushing of the capillary bed supplied by these dilated arterioles then follows, and it

is the distension of these minute vessels with bright arterial blood which is directly responsible for the flare.

Lewis (15) as reported by Shaffer (16) pointed out that any type of injury to the skin gives rise to the local formation of a histamine-like body (this hypothetical factor may be a single substance of a number of substances) which he calls "H-substance". When an acute injury is produced, a rapid release of "H-substance" follows. This results in a high local concentration of this factor with consequent full reproduction of the triple response. In chronic injury, on the other hand, such as is induced by mild burns or freezing, or in injury with long latency such as develops after exposur of the skin to ultraviolet rays, roentgen rays, or radium, th resp se is quite modified because "H-substance" is slowly relea ed and is simultaneously absorbed over a long period, so that high concentration is never present at any one time. It is, therefore, quite obvious that in injury of the skin res ting from roentgen ray or radium exposure, the triple response is only partially developed and usually consists of redness, slight edema and only a suggestion of a flare. In fact, if this injury is sufficiently mild, clinically, at least, only slight erythema may be present.

In 1927 Pohle (17) showed by means of a capillary

microscope that after administration of one arythema dose of roentgen rays the capillaries of the skin did not return to normal even after a period of 1 or 2 years.

Chrom (18) in 1936 injected a special strain of living bacillus intravenously. This was proceeded by a dose of x-ray varying from 10 to 75 r and ranging in time from a few hours to several days previous to the innoculation. He was unable to show any leukocytosis or decreased septicemia compared with the control animals and drew the definite conclusion that mild doses of x-ray have no effect upon the reticulo-endothelial system as to function or structure.

Stone and Aebersold (19) in 1937 reported work done with 200 and 1,000 kilovolt x-ray. In their summary they stated, "Experimental data has been collected on the recovery of human skin from roentgen and gamma irradiation.

"It is shown that if sufficient radiation be administered in a given time to produce the threshold effect, within the experimental limits investigated it makes no difference whether it is delivered in small doses with short intervals, or in larger doses w th longer intervals.

"It is further shown hat if the radiation be administered in a given period, i makes no difference, within the experimental limits investi ted, whether it is delivered

in long treatments of low intensity or in short ones of low intensity."

They showed also that the term "saturation method of dosage" is not accurate when a constant daily recovery factor is assumed. Since the amount of recovery decreases from day to day, it is necessary to know the correct recovery factor for every day.

Crowther (20) in a paper read in England in 1937 pointed out the wide variation in the effect of x-ray upon the individual cell. He mentions that a dose of only 40 r will kill half the individuals in a clutch of Calliphora eggs. A dose of 330,000 r is required to produce 50 per cent of deaths in a culture of Colpidium. Mitosis is inhibited in a tissue culture by a dose of 120 r, but 13,000 r are required to produce even a delayed lethal effect.

Barker (21) in 1938 felt that the evidence at that time demonstrated that the first and most important effect of x-ray was destruction of some of the infiltrating leukocytes, especially the lymphocytes which are most sensitive to radiation. With the destruction of the cells he theorized that the anti-bodies, ferments and other protective substances within the cell were liberated and made available to the local and the general circulation. The destruction of the

cells he believed was increased by the infiltration of leukocytes, and the phagocytosis carried out by them. The foregoing is his basis for the beneficial results obtained, with the early relief of pain. He further feels that this is the reason that x-ray is effective in small doses. Doses which are too small to have any effect on normal tissues. He enthusiastically states that he has shown recovery in cases of mastoiditis, hyperthyroidism, pelvic inflammatory diseases, thrombophlebitis, gas gangrene, carbuncles, furuncles, prostatitis, erysipelas, keloid formation, eczema, acne, actinomycosis, uterine hemorrhages, pneumonia, and relief of symptoms in allergic hay fever, hodgkins disease, leukemias and gonorrheal arthritis.

To determine the effect of therapeutic irradiation on the erythrocytes and hemoglobin of the circulating blood as revealed by the red cell count and hemoglobin determination and the effect of irradiation on the leukocytes as determined by the white cell count. Kornblum (22) and his associates in 1938 performed such studies before and after irradiation on 100 unselected patients with benign and malignant conditions, the latter predominating. Roentgen and radium irradiation as app-

lied therapeutically has no significant effect on the red cell count or the hemoglobin content of the blood. Anemia alone is not a contraindication to radiation therapy. Therapeutic irradiation tends to lower the leukocyte count. The greatest decrease occurs in the lymphocytes and then in the neutrophils, with the normocytes and the eosinophils being the least affected. There was no apparent relationship between the effect on the leukocytes and the part of the body treated, the amount of irradiation and the period of time during which the patient was irradiated. Kornblum concluded that from a practical point of view the effects of irradiation on normal blood as determined by the blood count, are of little clinical significance.

Shaffer (16) in 1940 reported some interesting work which he had done in attempting to determine the relationship of histamine to any substance which might be liberated when small doses of x-ray are given. He demonstrated this very interesting fact that if histamine were injected subcutaneously two to five days after a single or three daily doses of 125 r, both the flare and the wheal were smaller on the exposed skin than on the non-radiated site. He also produced a papule by

injecting a mixed catarrhal vaccine. He claims that the reaction around the papule was greater on the x-rayed surface than on the normal skin. This sounds somewhat contradictory to other experiments but actually on an average the radiated papules healed more quickly than the non-radiated ones. He gives the following theories to substantiate his laboratory findings:

"It is generally agreed that roentgen rays produce destructive effects on the cellular components of the skin. Theoretically this results in the release of "H-substance" and the products of protein decomposition.

"The present study of the behavior of the histamine wheal on roentgen rayed skin indicates that within forty-eight hours the permeability of the capillaries falls below normal; and this relative refractory state is maintained at a more or less constant level for a period of at least four days. From the evidence of investigators, it may be concluded that this refractory state eventually becomes more pronounced and may even become absolute after the roentgen ray reaction has passed its peak.

"The capillary dilatation which has been observed as early as four hours after a single roentgen

ray exposure proceeds in a series of waves for a period of several weeks, at least.

"The present study of the behavior of vaccine papules on roentgen rayed skin suggests that skin exposed to fractional doses of roentgen rays is in a state of latent if not actual leucocytic mobilization. The chemotactic influence of the products of protein decomposition, resulting from the action of the absorbed roentgen rays on cellular protoplasm, is the mechanism which offers the most probable explanation of this effect.

"It is possible that the phenomena described above are able to explain, in part, at least, the beneficial effects of roentgen rays on inflammed conditions of the skin and subcutis. A review of the literature dealing with this problem emphasizes certain facts and reveals that small doses of roentgen rays produce only a local effect on the tissue absorbing the radiation and that there is no direce effect on bacteria, antibodies, or enzymes in vitro. Most investigators agree that small doses of roentgen ray stimulate the reticuloendothelial system; but workers dealing with other phases of the inflammatory reaction are not in g neral agreement. These discordant results may possibly e accounted for on the

basis of widely divergent condition under which the different studies were persued. At any rate, such factors as non-specific pretein effect, non-specific mobilization of antibodies, increased blood flow, and the local destruction and mobilization of the cellular infiltrate, were emphasized by various authors as accounting for the favorable effects of roentgen rays in inflammatory states."

It is his opinion that the changes in permeability and dilatation of the capillaries resulting from the release of "H-substance" and also the changes in the cellular infiltrative reaction of the skin due to the release of decomposed proteins with its consequent non-specific shock-protein effect are able to account for the various phenomena in the experimental investigations described above. In this way, many apparently contradictory mechanisms and results can perhaps be brought into harmony.

Among the more recent experiments in the field of Roentgenotherapy we have a report by Soto, Brunchwig, and Schultz (23) in 1938. Their technique was to produce two skin lesions on the body surface of the same animal. One lesion was used as a control, the other was treated with x-ray. While they did not work with peritonitis it is interesting to note their conclusions, because any reaction in the skin might be quite similar to the peritoneal reaction. In a series of experiments based upon 105 rabbits and in which non-radiated control lesions were produced in every instance in the same animal that bore irradiated lesions the following conclusions were reached:

1. Moderate doses of 200 kv. x-radiation filtered by 1 mm. Cu plus 1 mm. Al, i.e. 600 r, reduce the severity of acute pyogenic infections in the skin and subcutaneous tissues but do not necessarily hasten the final healing of these lesions; indeed, in a small percentage of cases the irradiated lesions healed more slowly than the controls.

2. No evidence was obtained that the beneficial effects of x-radiation in inflammatory processes are due

to widespread destruction of leucocytes, especially lymphocytes, in the field with liberation of antibacterial ferments. In fact the exudate in the irradiated lesions was practically identical with that in the controls.

3. The optimum opportunity for beneficial effects is obtained when the irradiation is given shortly after the injection of organisms (within five hours) and decreases as the suppurative phase (abscess formation) of the infection becomes more prominent. Irradiation twenty four hours prior to bacterial injection did not inhibit the severity of the subsequent lesions; indeed, some of the lesions in such areas healed more slowly than the controls.

4. Evidence is presented to indicate that a factor in the mechanism of the beneficial action of irradiation is an effect upon the capillary bed of the field which results in more rapid absorption of soluble substances from the inflammed areas. This would permit of a less intense leucocytic mobilization necessary to cope with the infection.

Altmeir and Jones (24) were the first to report in the literature any experimental roentgenotherapy which dealt solely with peritonitis. They used full

grown rabbits and gave them approximately 90 per cent of a human erythema dose of x-ray (630 r) over the entire aspect of the abdominal wall. They used the following factors in treatment; voltage 200 kilovolts, rate 25 milliampers, filter 00.5 mm. of copper with 1 mm. of aluminum, skin target distance 50 cm. and intensity 42 roentgens per minute. The only complications were an occasional diarrhea.

With the rabbit under drop ether anesthesia they opened the abdominal cavity and the ~~intra~~peritoneal ~~fluid~~ (ادس) was examined before, and after the roentgen treatment. Twenty-four and, more ~~not~~ noticeably, forty-eight hours after the application of x-rays over the abdomen, the tissues were found to be markedly hyperemic, the vessels being dilated and engorged. The entire thickness of the anterior abdominal wall was two to three times greater than before. This thickness was principally due to the edema of the muscle layers, although the edema extended into the subcutaneous and subserosal layers of the abdominal wall as well. Little cellular infiltration was seen in the abdominal wall, but occasionally areas of eosinophilic infiltration were noted. The blood vessels were dilated and engorged. With the exception

araa.

of the marked edema and congestion, there was nothing remarkable in the sections studied.

At varying intervals after the roentgen therapy, these animals were given a single intraperitoneal injection of 3 cc. of a four to seven day brain broth culture of virulent bacteria. This culture was obtained in a fatal case of peritonitis and it consisted of Bacillus coli, aerobic nonhemolytic streptococcus, Bacillus pyocyaneus, Bacterium melaninogenicum, anaerobic streptococcus, and Clostridium sporogenes. Judging from a previous study, this culture was rather t typical of severe acute perforated appendicitis with peritonitis.

Percentage of Animals
Surviving bacterial injection.
80%

In summary Altemeir and Jones (24) found that a group of forty-two rabbits treated with approximately 90 per cent of an erythema dose was inoculated intraperitoneally with equal amounts of mixed highly virulent bacterial cultures. "It was found that the degree of protection of the treated animals rose sharply three weeks after irradiation and reached its maximum between the fourth and the sixth. A study of our experiments and a review of the literature have failed to explain the manner in which this protection is brought about."

"In the clinical series of patients with carcinoma of the rectum or rectosigmoid reported by Pratt, an interval of from four to six weeks elapsed between the time of roentgen therapy and operation. This was the period of time arbitrarily chosen as necessary to allow the patient to recover sufficiently from any deleterious effects of the x-ray. On the basis of our experimental work, this interval seems to have been wisely chosen since it also confers the highest degree of protection from peritonitis." They drew the following conclusions:

1. High voltage roentgen irradiation is valuable as an immunizing agent against experimental peritonitis in animals.

2. Furthermore, our observations suggest that preoperative irradiation is valuable in preventing postoperative peritonitis in human beings.

3. The dosage employed was 90 per cent of a human erythema dose.

4. The maximum degree of immunity in animals occurred from four to six weeks after the irradiation.

5. The manner in which this protective action is brought about is unexplained.

6. The experimental results presented in this paper further justify the continuation of preoperative roentgen irradiation in contemplated resections of the colon and rectum.

IV ORIGINAL WORK

The author, under the supervision of Dr. J. Dewey Bisgard, aided by Wilbur Overmiller, Oliver Horak and several other fellow students, wishes to present the results of the experiments which we have done here at the University Hospital. It is thought that they may be of some value as a basis on which to base some therapeutic rules. It is also fitting that we thank the Department of Radiology, the Department of Bacteriology, and the Department of Administration for their necessary supplies, cooperation and advice.

Only the most important and relevant experiments are included in this report. The results do not agree in many phases with the preceeding reports of other writers, but that is to be expected in any new field as is this one.

EXPERIMENT NO.I

The object of the experiment was to:

1. Determine the value of x-ray therapy in treatment of peritonitis in rabbits.

2. Establish the optimal amount of x-ray.

3. To decide the most effective time to give the treatments, relative to the inoculation.

The general procedure was to first obtain forty-eight rabbits, all of approximately the same weight, and from the same source of supply. These forty-eight rabbits were divided into eight groups of six rabbits each. The first group was used as controls. The second group was treated with x-ray one hour after inoculation. The remaining six groups were managed as follows:

Group A	12	weeks	between	last	treatment	and	inoculation.
Group B	6	"	"	"	"	"	"
Group C	4	"	"	"	"	"	"
Group D	1	"	"	"	"	"	"
Group E	3	days	"	"	"	"	"
Group F	24	hours	"	"	"	"	"

Each group was re-divided into three groups of two rabbits. Group 1 received 102 r each day for 6 days over the anterior abdomen. Lead shields were used to protect the thoracic and inguinal areas. The therapy machine

was set at 140 Kv.C. with a filter of ½ mm. ~~aluminum~~ copper and 1 mm. ~~copper~~ aluminum, port 15 x 13 cm., 15 milliamps, time 6 minutes, 102 r per treatment or a total of 612 r.

Group 2, received three identical treatments on the 2nd, 4th, and 6th day or a total of 306 r.

Group 3 received only 1 treatment on the 6th day or 102 r. The interval between treatment and inoculation was measured from the time of the last treatment.

The rabbits were inoculated intraperitoneally with a special strain of hemolytic colon bacillus suspended in 20 cc. of gastric mucin. All rabits including the controls were injected on the same date and time. The solution was thoroughly mixed so that each rabbit would receive the same amount. Sufficient number of organisms were used to cause death in an untreated rabbit within 9 hours. The sultures were carefully checked to be sure of the purity and virulence, as well as the hemolytic properties before each series of injections.

An autopsy on each rabbit was done immediately er death and a record was made of the condition of the pe itoneum and the fluid in the peritoneal cavity. Accurate re ording was also made of the survival time after inocula- ti n, percentage which survived, and the relation of the

amount of x-ray received in each group to the number of survivals. The results were:

Group	Lived	Died	Average Survival Time	Per Cent Survival
Group A	1	5	4½ hrs.	16.6%
Group B	0	6	5 hrs.	0.0%
Group C	1	5	5½ hrs.	16.6%
Group D	1	4	4½ hrs.	20.0%
Group E	4	1	6 hrs.	80.0%
Group F	3	3	7¼ hrs.	50.0%
Group G	0	6	7 hrs.	0.0%
Controls	0	6	6½ hrs.	0.0%

Graph No.2 on the following page illustrates the relationship between the total amount of x-ray given to the number of survivals.

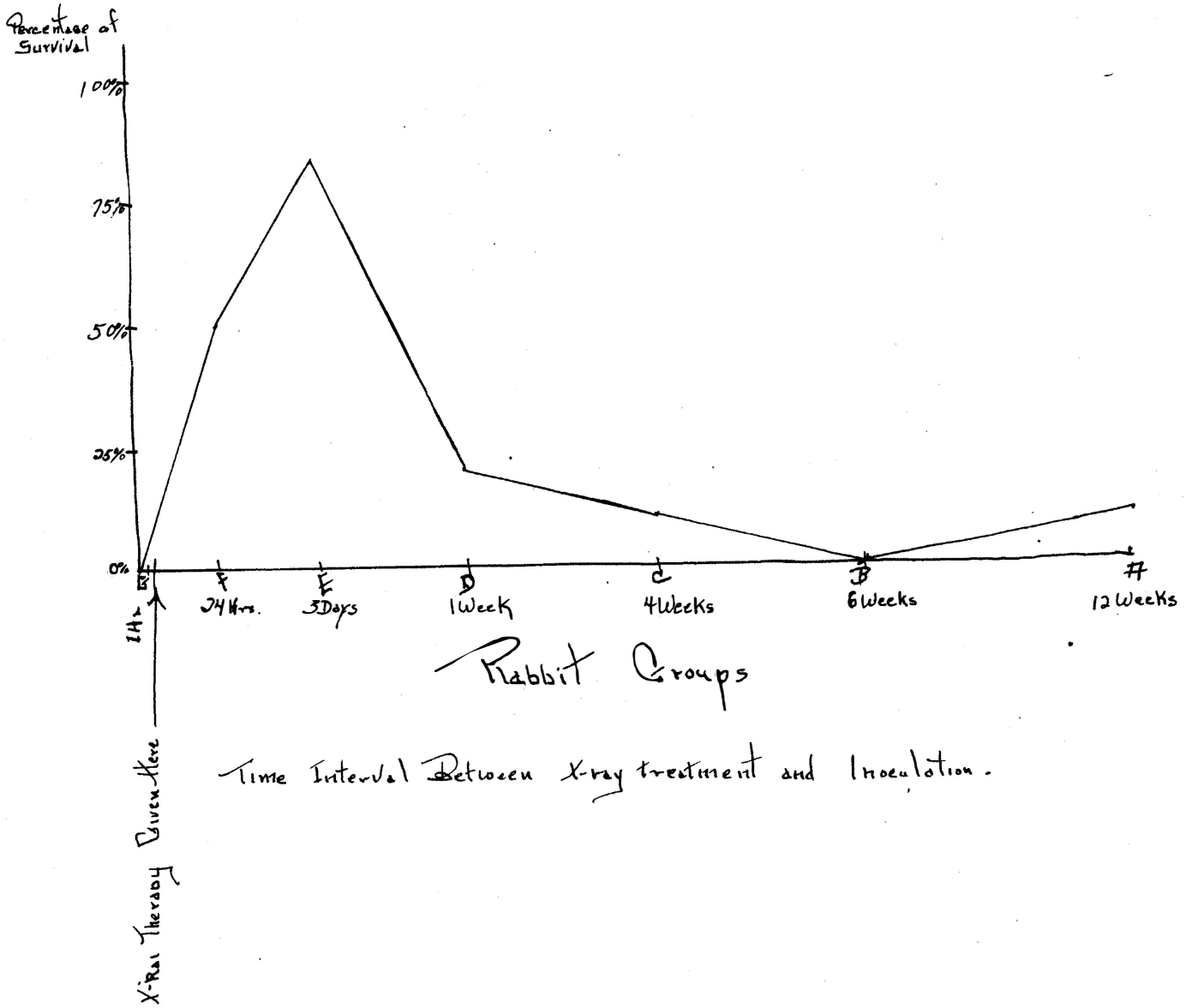
It illustrates clearly that there are no mathematical relationship between the total amount of x-ray and the results.

This helps to illustrate the fact that the time interval between inoculation and therapy is more important than the total amount of x-ray given.

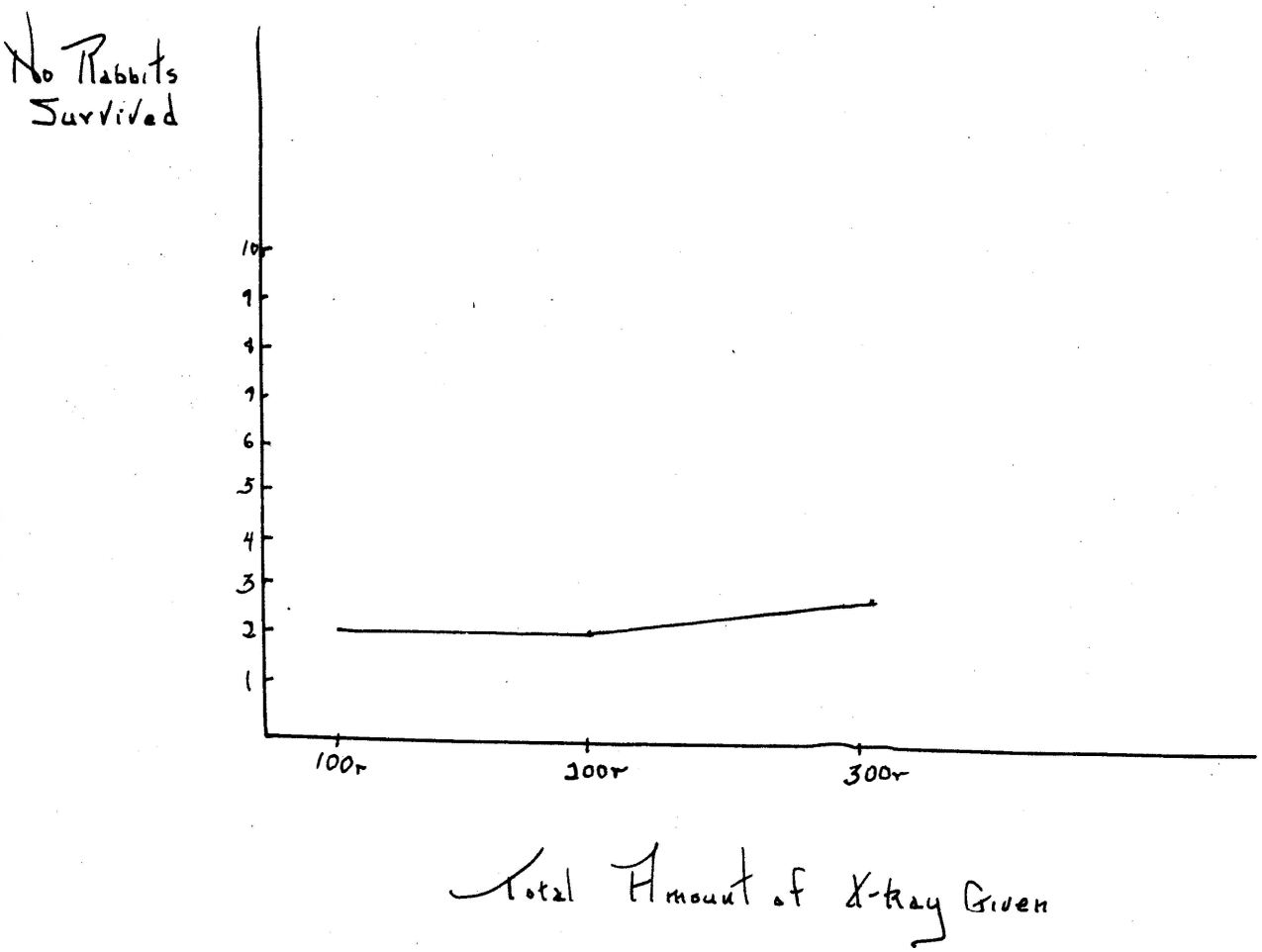
Conclusions:

1. Roentgenotherapy is helpful in cases of peritonitis produced in rabbits.

Graph No. 1.


Percentage of Survival
100%
75%
50%
25%
0%
X-Ray Therapy Given Here
24 Hrs.
3 Days
1 Week
4 Weeks
6 Weeks
12 Weeks
Rabbit Groups
Time Interval Between X-ray treatment and Inoculation.

Graph No. 2


No Rabbits Survived
10
9
8
7
6
5
4
3
2
1
100r
200r
300r
Total Amount of X-ray Given

2. The most effective time to administer x-ray is about two or three days before inoculation. (See graph No. 1)

3. The total dosage of x-ray is not as important as the time interval between the last dose and the inoculation. (See Graph No. 2)

EXPERIMENT NO. II.

The following experiment was done at a later date to verify the previous work. The cultures of Hemolytic Colon bacilli were rechecked for their virility and purity. The same procedure was used as in the previous experiment. The colon bacilli were suspended in 20 cc. of gastric mucin and given intraperitoneally, as before. Twelve rabbits were used this time, and divided into the following groups.

Group I. Controls, 3 rabbits. They received no therapy.

Group II. 2 rabbits which received two 103 r doses of x-ray in consecutive days. The last treatment was 3 days previous to the inoculation.

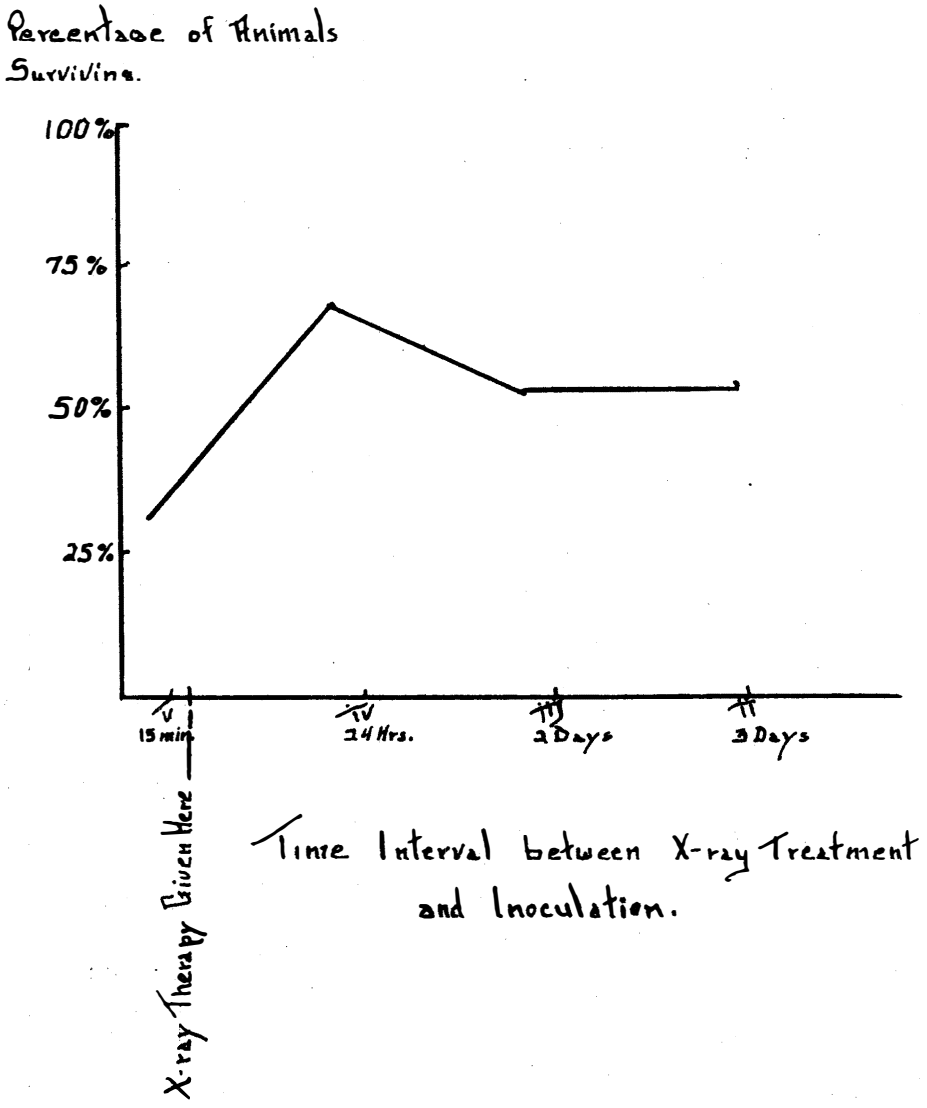
Group III. 2 rabbits received one dose of 103 r ~~three~~ two days previous to the inoculation.

Group IV. 3 rabbits received one dose of 103 r 24 hours before the inoculation.

Group V. 2 rabbits received one dose of 100 r 15 minutes after the inoculation.

Results:

	Lived	Died	Average Survival Time	Per Cent Survival
Group I Controls	0	3	8 hrs. 45 min.	0%
Group II	1	1	5 hrs. 30 min.	50%
Group III	1	1	7 hrs. 10 min.	50%
Group IV	2	1	5 hrs. 10 min.	66%
Group V	1	2	5 hrs.	33%



· Group I Controls - all died.

EXPERIMENT NO. III

This experiment was done to determine the value of gastric mucin in our peritonitis experiments. The hemolytic colon bacilli were suspended in gastric mucin in the previous experiments because it was thought that it would speed up the reaction, and increase the absorption. Seley (25) was able to show a definite increased mortality by using gastric mucin in producing experimental peritonitis in cats.

The procedure was to take two groups of three rabbits. The first group was given a suspension of the hemolytic colon bacilli in gastric mucin intraperitoneally. The second group received the same number of bacilli suspended in normal saline. At the same time two controls were injected with the same amount (20 cc.) of gastric mucin which contained no bacilli.

Results:

	Lived	Died	Average Survival Time	Per Cent Survival
Group 1.	0	3	6 hrs. 10 min.	0%
Group 2.	0	3	9 hrs. 15 min.	0%
Control (Mucin only)	2	0	No signs of any illness.	100%

It is quite evident that gastric mucin intraperitoneally seems to enhance the action of colon bacilli in peritonitis. This alone may account for the severe reaction following perforation of a peptic ulcer.

EXPERIMENT NO. IV

As has already been discussed the three main causes of death in acute peritonitis are: 1. Septicemia, 2. Toxemia, 3. Shock. The following experiment was done with the purpose of determining if injecting a sterile toxin from the Colon bacilli used in the previous experiment would be lethal and if so would x-ray have any beneficial effect.

Part 1.

Four rabbits were injected intraperitoneally with a heat-killed suspension of the special hemolytic colon bacillus used in the previous experiments. The toxin was prepared by suspending an estimated lethal dose of living organisms in normal saline. It was necessary to suspend the bacilli in normal saline because heat causes coagulation of the gastric mucin. The container was then immersed in a beaker of boiling water for 12 minutes. Several samples were taken to be certain that the solution was sterile. A sample of the solution was then taken at the time of injection and found to be sterile.

Results: All four rabbits died. Average

survival time was five hours and ten minutes.

Part 2.

Five rabbits were given 103 r. in one treatment in exactly the same manner as the previous experiments. Forty-eight hours later they were given intraperitoneally 20 cc. each, of the heat-killed toxin, prepared exactly the same as above. Two controls were also injected at the same time.

Results: Three out of the five animals lived.
Both controls died.

The experiment was repeated at a later date. The same colon bacillus culture was used. It was rechecked for its purity and virulence and found to be as lethal as previously.

Six rabbits were x-rayed identically as before - 140 Kv., 15 Ma. and filter of ½ mm. Al and Cu. Forty-eight hours later they were injected with 20 cc. normal saline suspension of the heat-killed organisms. The solution was checked at the time of injection and found to be sterile. Each rabbit as before received 20 cc. of the solution intraperitoneally. The results were:

	Lived	Died	Average Survival Time	Per Cent Survival
X-rayed Group	4	2	8 hours 45 minutes	66%
Controls	0	2	8 hours 30 minutes	0%

All of the animals which died in this experiment were autopsied as soon after death as possible. In all but one the large bowel was distended. The only thing of any significance found was that in the rabbits which had been x-rayed the peritoneum was less inflammed and that there was less fluid in the peritoneal cavity.

EXPERIMENT NO. 5

The question now was whether the function of x-ray in the previous experiments was local or systemic.

To determine this, rabbits were given the usual treatment of x-ray (103 r.) in the identical procedure used before. Forty-eight hours later they were bled. The blood was allowed to coagulate. The clots were broken up and then centrifuged. An average of 22 cc. of serum per rabbit was obtained in that manner. The serum was then mixed with a lethal dose of a normal saline suspension of the hemolytic colon bacillus and injected intraperitoneally, intravenously, or subcutaneously into another rabbit. A control was run in each case to be certain of the virulence.

	X-rayed Serum		Normal Serum	
Date	Lived	Died	Lived	Died
10-23-41	2 (I.P.)	0	0	1 (I.P.)
12-11-41	2 (I.P.)	0	0	1 (I.P.)
12-15-41	0	1 (I.V.)	0	0
12-17-41	1 (I.V.)	1 (I.V.)	1 (I.V.)	0
1-3-42	2 (Subcut.)	0	1 (Subcut.)	0
1-16-42	1 (I.P.)	0	0	1 (I.P.)
Totals	8	2	2	3

Date	Controls Lived	Died
10-23-41	0	1 (I.P.)
12-11-41	0	2 (I.P.)
12-15-41	0	1 (I.V.)
12-17-41	0	1 (I.V.)
1- 3-42	1 (Subout.)	1 (Subout.)
1-16-42	0	1 (I.P.)
Totals	1	7

Per Cent Survivals

x-rayed serum 80%

normal serum 40%

controls 12½%

EXPERIMENT NO. 6

Since the previous experiments indicate that there is a definite advantage in administering serum from x-rayed rabbits into other rabbits who have been inoculated with either a living or a killed suspension of a strain of hemolytic colon bacilli, it was thought that perhaps it might have a similar action against other toxins. Diphtheria toxin was obtained from the Eli Lilly Laboratories at Greenfield, Indiana. Its specifications were: 42 Lf units, M.L.D. 1/2100, and L+0.035. This toxin was diluted 1:1050 which made it equivalent to 2 M.L.D. per cc. of solution.

The rabbits were divided into four groups. Each rabbit was given 4 M.L.D. per Kilo of body weight. The toxin was mixed with the serum and allowed to stand for one hour before injection. The first group received serum from normal rabbits, plus diphtheria toxin as stated. The second group received serum from x-rayed rabbits plus diphtheria toxin as stated. The third group received x-ray only, plus the diphtheria toxin as stated. The fourth group were used as controls, receiving no therapy, only the diphtheria toxin as stated. In all cases the toxin-serum solution was given intraperitoneally.

The results were:

Date of Injection	Normal Serum Alive	Normal Serum Dead	X-rayed Serum Alive	X-rayed Serum Dead
1-19-42	0	0	0	0
1-21-42	0	0	0	0
1-29-42	0	2	0	2
2-3-42	0	2	0	0
2-13-42	0	0	0	0
Totals	0	4	0	2

Date of Injection	X-ray Only Alive	X-ray Only Dead	Controls Alive	Controls Dead
1-19-42	0	0	0	2
1-21-42	1	2	0	2
1-29-42	0	0	0	0
2 -3-42	0	0	0	0
2-13-42	1	5	0	3
Totals	2	7	0	7

Per Cent Survivals

Normal Serum 0%

X-rayed Serum 0%

X-ray Only 22.2%

EXPERIMENT NO. VII

This experiment was done exactly the same as Experiment No. VI, except that the amount of diphtheria toxin was reduced to one-half of the previous dosage. Therefore each rabbit in this group was given 2 M.L.D. per kilo. of body weight.

The results were:

Date of Injection	X-ray only		X-rayed Serum		Controls	
	Lived	Died	Lived	Died	Lived	Died
2-23-42	5	3				4
3-4-42			3	2		2
3-22-42			1	4		2
Totals	5	3	4	6	0	8

Per cent survival:

X-rayed Serum 40 %

X-ray only 62½ %

Controls 0 %

Autppsies were done on all rabbits immediately after death. The only significant finding was that in all cases there were numerous sub-mucosal hemorrhages on both sides of the pylorus. The stomach was full of food material and the pyloric valve was contracted.

V. SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS.

A survey of the literature on experimental roentgenotherapy and experimental peritonitis has been presented. All of this work has been done on animals. There is a wide variation of opinions as to the actual functions of X-ray. Some authors claiming little or no local effect of mild doses of X-ray, while others maintain that it produces changes in the cells and tissues. The most recent and best established theory is that forty-eight to seventy-two hours after exposure to x-rays the tissues show a definite hyperemia and leukocytic infiltration. There is probably a release of "H" substance which may have some antigenic properties. It is also claimed that X-ray in mild doses will decrease permeability of the capillaries.

A series of seven experiments done here at the University Hospital has been presented. One hundred and fifty-six rabbits were used. The effect of mild X-ray doses on experimental peritonitis and toxemia cases was found to be beneficial.

Serum taken from x-rayed animals was found to be of some value but not as effective as direct administration.

CONCLUSIONS

1. Roentgenotherapy in mild doses has a definite prophylactic value in cases of experimental peritonits or toxemia.

2. Mild doses of X-ray causes a liberation into the blood of some generalized antitoxin which is probably the "H" substance.

3. The period of greatest efficiency of the X-ray therapy is from forty-eight to seventy-two hours before inoculation.

Remarks;

While it is often ridiculous to prognosticate it is thought by the author that in the future, patients will receive X-ray therapy previous to any operation which shows a high incident of peritonitis. Also blood donors will be given small amounts of X-ray before giving blood for a transfusion.

It is also suggested that X-ray may have a definite value in preventing shock by decreasing the capillary permeability. This may be true also in burns where there is a great loss of serum from the subcutaneous capillaries.

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