THE PRODUCTION OF EXPERIMENTAL TYPHOID FEVER IN THE GUINEA PIG WITH AN IN VIVO PREPARED TOXIC FILTRATE OF B. TYPHOSUS.*

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PLATES 21 TO 23.

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The experiments of Grunbaum (1) and of Metchnikoff and Besredka (2) have demonstrated that when Bacillus typhosus is introduced into anthropoid apes, certain evidences of human typhoid fever can be reproduced. Positive blood cultures, agglutination reactions, fever, lesions of Peyer's patches and other features of the disease have been reproduced in this type of animal. The experiments carried out with this organism upon the smaller and more practical laboratory animals have, however, as a whole, proven unsatisfactory. Although a septicemia, peritonitis and death can be readily produced in such smaller animals, the resultant picture bears little if any relationship to the human disease.

Besredka (3) has found that smaller animals primarily prepared by feeding bile are vulnerable to the ingestion of typhoid bacilli and present a more protracted infection with certain features allied to human typhoid. More recently Sedan and Herrmann (4) have employed the injection of typhoid bacilli into the subconjunctival tissues. They have produced by this means a continued fever, diarrhea, tumefaction of Peyer's patches and certain other features analogous to human typhoid although the microscopic study does not appear identical with that found in the human infection. Gory and Dalsace (5) have also reported typhoid infection in the guinea pig, employing the method of Sedan and Herrmann. Although they state that the human disease has been duplicated, a review of their pathological study shows that multiple abscesses were produced in the liver, spleen and the lymphoid tissues of the peritoneal cavity including

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Peyer's patches. They also specify that acute cholecystitis was almost constant and acute pericarditis was at times present. Their results indicate that through the method employed they prolonged the duration of the usual septicemia, permitting thereby the formation of widespread acute exudative lesions. Such lesions do not conform with the host response characteristic of the human disease, the histopathology of which was first most fully described by Mallory (6).

In addition to numerous transmission experiments performed with the organism proper, extensive experimentation has been carried out with the toxic products. Evidence has been advanced in support both of its endotoxic and ectotoxic nature. A great variety of methods have been employed for the purpose of obtaining a specific toxin. A general review of the literature appertaining to the toxin of the organism is to be found in the work of Gay (7).

Persistent difficulty has been encountered in procuring a true specific toxin for many microorganisms, especially those regarded as endotoxic in nature. In this connection, Duval and Hibbard (8) have reported the effects produced in animals by the injection of a toxic material obtained from the streptococcus of scarlatina by means of an *in vivo* process. In their method the supply animal was first immunized by several injections of the streptococcus administered at weekly intervals. It occurred to us that the employment of the animal host in some intermediary capacity may be essential in the production of a toxic material more closely allied to that demonstrated in the typhoid toxemia of man.

When the typhoid bacillus is introduced into the peritoneal cavity of normal guinea pigs an exudative peritonitis is produced. In this conflict of the invading microorganism and the animal host the usual resultant features of an acute inflammatory reaction are found. It appeared likely that in this field of activity in which many factors both of host and invader have been put into action, a toxic substance might be produced which when injected into animals would yield a different character of response than that obtained by the living microorganism.

The experiments herein reported demonstrate the results obtained by the injection into guinea pigs of the toxic factor procured through this *in vivo* method.
A peritonitis was produced in guinea pigs by the inoculation of cultures of *B. typhosus*. The virulence of the strain was exalted by passage through several generations of guinea pigs so that within 7 to 10 hours after injection the animals became very sick and the abdomen distended with exudate. The animals were then sacrificed and the fluid was withdrawn from the peritoneal cavity. The exudate varied from a slightly smoky yellow liquid to one of a cloudy, flaky character, rich in cellular elements. This material was diluted to approximately four times its volume with sterile water and filtered through an N Berkefeld filter. The resultant bacteria-free filtrate was employed for animal injection. It was found preferable to employ freshly prepared filtrate at each interval injection.
Twenty-four guinea pigs have thus far been employed. The protocols of six representative animals of this group have been selected. Of these six, two received the injections subcutaneously, two intraperitoneally and two intracardially. All six animals died in approximately 2 to 4 weeks. The autopsy findings and microscopic study are described under pathology.

Experiment 1.—Two guinea pigs weighing approximately 250 gm. were given four subcutaneous injections of in vivo prepared toxin as follows: Each received 4 cc. as a primary injection, 3 cc. 3 days later, 5 cc. on the 5th day and 4 cc. on the 12th day. One of these animals died on the 17th day and the other 11 days later. The temperature and leucocytic counts of each are shown in Chart 1.

As can be seen, subsequent to the injections there usually occurred a rise in temperature and a drop in the leucocytic count. One of the animals showed a drop of the count to 500 cells and the other to 3000 cells per c. mm.

Both animals gradually lost in weight and their appetite became poor. No other clinical manifestations were observed.

Experiment 2.—Two guinea pigs were given four intraperitoneal injections of in vivo prepared toxin on the same days as those of Experiment 1 but the following respective amounts were administered, 2 cc., 2 cc., 3 cc. and 4 cc.
The febrile and leucocytic reactions in these two animals were quite consistent and closely parallel. The leucocytic count in both instances dropped to nearly 2000 cells per c. mm. with frequent counts of around 3000 as can be seen in Chart 2. These animals lost in weight, one showing considerable emaciation. One animal died on the 22nd and the other on the 23rd day following the inoculations. No diarrhea was observed in either animal.

**Experiment 3.**—Two guinea pigs were inoculated intracardially with the in vivo prepared typhoid toxin. They each received on the 1st day, 1 cc. of the toxin, on the 3rd day, 1 cc. of the toxin, on the 5th day, 2 cc., and on the 12th day, 1.5 cc. Their febrile response and leucocytic decline were marked and followed constantly the injections. The fever rose as high as 106.4°F. for one animal and 105°F. for the other. The leucocytes dropped below 3000 per c. mm. on several occasions, the original counts before injection being approximately 10,000 cells per c. mm. One of these animals died on the 13th day and the other
on the 15th day. The reactions by this route of inoculation were sharper (see Chart 3) and the animals died more quickly.

It can be seen from a review of the charts of these six animals that as a whole the clinical records are analogous in character. With each injection of the toxic material, irrespective of the route of administration, a rise of temperature and a drop of the leucocytes occurred. The febrile response was of a transient character lasting for 2 or 3 days after each inoculation. The abatement of the fever is attributable most likely to the fact that no continued toxemia or accumulative toxic action existed since no living virus was injected. In the instance of the leucocytes, however, after the first injection of toxin the total count seldom returned to the normal; the leucopenia continued and became accentuated subsequent to each inoculation. It would appear that the particular factors or mechanism essential for the production of leucopenia was continued over a longer period of time than the phenomena producing the fever. In no instance was diarrhea observed and aside from loss of appetite and weight and progressive weakness together with the leucocytic and febrile response no other clinical features were discernible.

Pathology.

The postmortem examination of the animals in which death followed the typhoid toxin inoculations showed as a whole the same general gross picture. In certain of the animals the changes were more accentuated than in others but this variation was only in degree or extent and not in the character of the lesions.

At the inoculation site of the animals injected subcutaneously there was found a congested and edematous area about 2 to 3 cm. in diameter. Small hemorrhagic extravasations were at times noted.

The abdominal cavity showed the lymphatic glands to be greatly enlarged and in some of the glands marked congestion and hemorrhage were present. (See Fig. 1.)

Spleen.—This structure was usually of a deep red color and moderately increased in size. The consistence was soft and the pulp stripped off readily, although two of the six spleens were quite firm. No evidences of miliary abscess formation or areas of necrosis were found.

Intestines.—The examination of the intestinal tract showed throughout a
marked enlargement of all lymphoid structures. The solitary follicles protruded well into the lumen and were frequently congested. The Peyer's patches were greatly elevated (see Figs. 2 and 3), often reddened and at times showed slight ulceration. Occasionally a marked necrosis and ulceration of the patch occurred. The fecal content in these areas was fluid in character but no blood was observed.

Liver.—This organ was enlarged, congested and friable and of a deep red color. Scattered throughout the structure were found yellow focal areas varying in size from 1 to 3 mm. The gall bladder showed nothing of note.

Kidneys and Adrenals.—These structures were somewhat swollen and the vessels showed considerable congestion. Evidences of yellowish discolorations suggestive of degeneration were sometimes found.

Bone Marrow.—This was more deeply reddened than normal marrow of the guinea pig.

Heart and Lungs.—No gross lesions were observed. In no instance was pneumonia found present.

Nothing else of note was observed in the gross study of other structures of the body.

Microscopic.—The voluntary muscle in the region of the subcutaneous inoculation showed hyaline or waxy necrosis of this structure. Scattered wandering monocytes or "endothelial" cells were seen about the necrotic muscle. The enlargement of the lymphatic glands especially of the peritoneal cavity and of the solitary follicles and Peyer's patches was found to be due to the hyperplasia of the lymphoid structures and to the accumulation of "endothelial" cells. Many "phagocytic cells" of Mallory were noted in which the engulfed elements consisted of portions of lymphoid cells, nuclear fragments and at times erythrocytes. In certain of the lymphoid structures marked congestion, hemorrhagic extravasations and areas of necrosis were seen. Some of the Peyer's patches showed a loss of the mucosa and necrosis of the cellular elements of the enlarged nodule with destruction of the muscularis mucosae. (See Figs. 4 to 6.) Sections of the spleen demonstrated marked congestion of the pulp and in some of the animals, hemorrhages were found in this structure. Escape of the hemoglobin was noted in masses of the erythrocytes presenting thereby shadow or phantom corpuscles. (See Fig. 7.) Scattered throughout the splenic structure but particularly in the pulp were endothelial cells many of which showed phagocytosis especially of the shadow red cells.

The liver sections revealed degenerative changes including areas of focal necrosis. These necrotic areas showed no special zonal location occurring centrally or in the portal areas or extending through both zones, and they varied in their size and extent. In some of these areas hemorrhage was present and many of the extravasated cells were located within the protoplasm of the phagocytic cells. The smaller areas of focal necrosis showed replacement of the hepatic cells by the endothelial type of cell. (See Fig. 8.)

The kidneys showed congestion of the vessels and degenerative changes in the
epithelium of the uriniferous tubules. The bone marrow showed marked congestion and at times hemorrhagic extravasations. Nothing noteworthy was found in other structures examined. It is of interest to note that no polymorphonuclear neutrophils or other elements of an acute exudative inflammation were found in the various lesions studied.

Controls.—In a previous article (9) we reported parallel experiments in which B. coli communior was employed. Fifteen guinea pigs were used and the results obtained therein were unlike those obtained with B. typhosus. Evidences of toxemia with degenerative and hemorrhagic lesions were found. Although death was produced by the toxin of this microorganism, the gross and microscopic aspect did not resemble the human typhoid lesion.

In this connection, the paratyphoid epizootics occurring in the guinea pig should be mentioned. The lesions of this infection as observed by one of us (10) and as studied by Howell and Schultz (11) do not resemble the lesions herein described. Work upon the effects in guinea pigs of the toxic material prepared in a similar manner from paratyphoid bacilli is under way.

DISCUSSION.

In general microorganisms belonging to the so called endotoxic group do not yield in vitro a satisfactory specific toxin. This has greatly interfered with the progress of the study of the true nature and effects of such microorganisms. For example, the preparation of a specific toxin from the streptococcus considered as the cause of scarlet fever has been attended with difficulties. Dochez (12) considers that this microorganism contains at least two varieties of poisons. Duval (8), on the other hand, employing his in vivo method regards the toxin procured by him from the streptococcus of scarlatina as the true specific toxin of this microorganism. In the instance of the typhoid bacillus, the same general difficulty of obtaining a specific poison has been encountered although numerous and diverse methods have been applied.

A definite toxemia is manifestly present in typhoid fever and the toxin produces in the human host a specific pathological picture. It is not improbable that such a microorganism forms its specific
toxin only when invading its natural host, in other words during its function of pathogenesis. A different or more complete biological process may be evolved in vivo as contrasted with in vitro activity. Again the somatic cells of the invaded host may play some essential rôle in the production of the specific pathogenic toxin. Because of these possibilities of a differential nature of such toxins, the in vivo method was employed. It is believed that the toxic material obtained through this process closely simulates in its action on the inoculated host the activities of the typhoid toxin as evidenced in the human disease.

SUMMARY.

When the typhoid bacillus is injected into the peritoneal cavity of guinea pigs acute peritonitis and death are produced. The character of the exudate is variable as to the elements present but is usually of a serous type with slight clouding due to the presence of polymorphonuclear neutrophils, mononuclear cells and bacteria. When the Berkefeld filtrate of this exudative material is inoculated into normal guinea pigs either subcutaneously, intraperitoneally or intracardially, the character of response obtained on the part of the host is quite at variance with that produced by the inoculation of the living typhoid bacillus. A febrile reaction and marked leucopenia, as a rule, are persistent and are accentuated after each injection the latter often reaching below 1000 cells per c. mm. There is a loss of weight of a variable extent in all animals and in some the emaciation is extreme. The animals were given four such inoculations and all succumbed in from 2 to 4 weeks. The intracardiac route produces death more quickly and the reactions are more clear-cut when this route is employed. At autopsy a general tumefaction and congestion of the lymphoid structures more especially of the abdominal cavity are found. Peyer's patches and the solitary follicles of the intestinal tract are likewise involved and in some of the patches slight ulceration is noted; occasionally, there occur extreme ulceration and necrosis of the patch. The spleen is enlarged and usually softened. Microscopically, marked endothelial cell proliferation is noted especially in the lymphoid structures and in many instances the phagocytic cells of Mallory are found. These cells include within their
cytoplasm elements of the surrounding structures. In the spleen there are present congestion, and hemorrhages with many "shadow" red blood cells. Phagocytosis of the red cells by the endothelial cells is present. In the liver, areas of focal necrosis are found and phagocytic cells are seen. In the animals inoculated subcutaneously, localized degenerative changes are observed especially in the muscular structures.

From these results it can be seen that the reactions and injury of the animal body by the toxic filtrate employed, are quite similar to the changes produced by the specific toxin in human typhoid fever.

CONCLUSION.

During the activity of peritonitis produced in the guinea pig by means of Bacillus typhosus, there is formed in the exudative material a filtrable toxic moiety which when inoculated into normal animals of this species, produces certain of the clinical phenomena and a pathological picture simulating that of human typhoid fever.

BIBLIOGRAPHY.


EXPLANATION OF PLATES

PLATE 21.

Fig. 1. Lesions of mesenteric lymph glands. These structures are greatly increased in size; congestion and hemorrhage are at times present.
Fig. 2. Specimen of a portion of the ileum showing Peyer's patch with enlargement and elevation of the structure and early ulceration at the upper portion. (Enlarged for detail.)

Fig. 3. Lesion similar in character to that in Fig. 2, with somewhat more marked swelling.

Fig. 4. Lesion of Peyer's patch showing marked swelling of the patch, and in certain areas, there is a loss of the epithelial lining. (Low power.)

PLATE 22.

Fig. 5. Cellular elements of swollen Peyer's patch. Cells of the phagocytic type of Mallory, which contain lymphoid cells and nuclear fragments, can be seen. (High power.)

Fig. 6. High power of lesion of the lymphatic gland, demonstrating the character of the proliferating cells.

PLATE 23.

Fig. 7. Section of spleen showing congestion and hemorrhage. The erythrocytes for the most part are of the "shadow cell" type (loss of hemoglobin) and some are engulfed by phagocytes.

Fig. 8. Area of focal necrosis in the liver. The hepatic cells have been replaced by the endothelial cells which have phagocytized fragments of red blood cells and nuclear portions. (High power.)
(Harris and Larimore: Experimental typhoid fever.)
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