STUDIES IN THE PATHOGENESIS OF EXPERIMENTAL DYSENTERY INTOXICATION

PRODUCTION OF LESIONS BY INTRODUCTION OF TOXIN INTO THE CEREBRAL VENTRICLES

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In the course of studying the pathogenesis of some visceral lesions associated with shock of various origins (1), we were impressed by the wide distribution of these lesions. This could not be explained by local organ characteristics but only by a process, systemic in nature, and centrally controlled. The anatomic resemblance of the gastrointestinal alterations in shock to those in bacillary dysentery and many other “toxemias” led us to investigate the pathogenesis of these lesions in animal experiments (2–6). These suggested that the lesions were the result of vasomotor compensatory reactions elicited as part of the homeostatic response to the toxin and thus centrally regulated. The present study was undertaken to further test the hypothesis that Shiga toxin produces its main effects through its action in the central nervous system. This was done by bringing the toxin into direct contact with the lining of the cerebral ventricular system.

In our previous studies of various visceral lesions occurring in shock, a morphological analysis indicated that the early changes were focal and that the larger lesions represented fusion of such focal alterations, on which secondary processes were superimposed; i.e., local infection, organ motility, local secretion, etc.

The tissue changes in Shiga intoxication showed a striking uniformity in proximo-distal distribution within a species while varying from one species to another (2); i.e., this distribution seemed to be species-specific. Likewise, the site of the earliest lesions within the layers of the intestinal wall showed a similar uniformity which correlated with the location of arterio-venous anastomoses in either the mucosa or submucosa of the bowel wall (3). When these anastomoses were located in the submucosa, the earliest changes were noted in the mucosal layer. When these short circuits were found in the mucosal layer, the earliest abnormalities were seen in the submucosa. With spread of the lesions and the addition of secondary factors, these significant differences were obscured.

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Pathogenesis of Dyentery Intoxication

Topical application of the Shiga toxin to the intestinal mucosa, even for a period of hours, produced no local alterations but only the usual remote lesions (2). In addition, the formation of visceral lesions as well as concomitant physiological changes were prevented by pretreatment of the animals with ergotamine tartrate and etamon chloride (3). The pharmacological studies indicated that the toxin was effective through some mechanism which was located in the central nervous system.

The implications of the pharmacological studies were further investigated by cross-circulation experiments (6) in which the blood supply of the brain of one of a pair of dogs was derived from the torso of the other of the pair. Circulation of the toxin in this manner resulted in typical lesions and physiological changes in the dogs in which the toxin was circulated through the brain. The results of these experiments indicated adequate physiological, if not anatomical separation of the two circulations. Nevertheless, the possibility remained that some toxin might have entered the torso of the dog which developed lesions even though we had used minimal amounts of toxin to avoid this. In the present investigation we further reduced the dosage and introduced the toxin into the ventricular system and thus into direct contact with the brain substance via the cerebrospinal fluid.

The permeability of the blood-brain barrier is such as to permit rapid exit of foreign substances into the brain only in certain areas (7). These include the posterior lobe of the pituitary, tuber cinereum, area postrema, epiphysis, paraphysis, the wall of the optic recess and the eminencia saccularis of the hypophyseal stem. In these areas, dyes such as trypan blue stain brain substance after intravenous injection; the rest of the brain is unstained (8). Large molecules, i.e. proteins with a range of molecular weight to a level of 100,000, have also been found to preferentially use these areas as points of exit from the capillary circulation. It seems safe to conclude, therefore, that in these regions the blood-brain barrier is absent or reduced. Absorption from the subarachnoid space and ventricles seems to be more diffuse and certainly the brain may be more readily and rapidly stained with trypan blue by introduction of the dye in these areas (9) which seem to be in free communication with the substance of the brain. We, therefore, devised a method of introducing Shiga toxin into the third ventricle by inserting a plastic tube through the fourth ventricle into the iter up to the level of the corpora quadrigemina.

Materials and Methods

In one experiment we used the same toxin as that employed in the original experiments. In the remainder we used a purified Shiga toxin kindly supplied by Dr. W. T. J. Morgan of the Lister Institute of Preventive Medicine, London. The material consisted of a conjugated polysaccharide-protein complex with a molecular weight in the 20,000 range which possessed a LD₅₀ of 80 μg. in mice weighing 18 to 22 gm. and induced formation of Shiga "agglutinins" in rabbits on the injection intravenously of 1 μg. while inducing a minimum significant rise of temperature (0.6°C./kg. rabbit) in the intravenous injection of 0.002 μg. (10). The materials
were dissolved in sterile isotonic saline forming an opalescent solution. When not used they were kept under refrigeration and brought to room temperature before injection.

Hematocrit determinations were made by the method of Wintrobe (11). The hematologic studies were done with standard pipettes and chambers. Blood smears were stained with Wright's stain. Blood clotting was prevented by moistening the syringe with heparin; no other treatment was found necessary. Blood, so treated, was used to determine plasma chloride content by the method of Van Slyke; sugar was determined by modification of the Folin-Wu technique (12).

**Procedure**

The data reported here represent the results of nine experiments on dogs in six of which the toxin was successfully injected into the area of the third cerebral ventricle.

The animals were prepared as follows: After a preliminary control blood specimen had been drawn, the dogs were anesthetized with intravenous nembutal. They were placed prone and the head allowed to fall over the edge of the operating table so as to increase the space between occiput and spine. The skin was incised over the line of the occipital ridge which was exposed to within 2 cm. of its lateral ends. Under careful hemostasis the platysma and muscles extending from the transverse process and spine of the second cervical vertebra and the occiput were transected. The deeper muscle layers were more readily separated from the occiput by peeling the periosteum from the surface of the bone. This was extended to the edge of the foramen magnum and on to the surface of the lamina of the vertebral body. This permitted an almost bloodless exposure; such bleeding as occurred was stopped by pressure packing before proceeding. In this matter the diamond shaped space between the occiput and first vertebra was exposed. In dogs weighing from 15 to 20 kg., this area usually was about 3 to 4 mm. on each edge; the widest diameter was about 8 to 10 mm. in width, the narrowest 4 to 5 mm.

At this point in the procedure, another “control” specimen was taken. The fibrous covering of the above hiatus was then incised. A variable amount of clear fluid escaped. When this ceased, the opening was enlarged to the edge of the occiput. Usually this permitted clear visualization of the surface of the medulla and the caudal tip of the tonsil of the cerebellum. These formed an acute angle in which a fine mesh of opalescent fibers was visible. These were avascular since there was no bleeding when the plastic tube was inserted through this area in the midline thus passing over the floor of the fourth ventricle into the iter. On the basis of trial experiments, the tube was inserted to a distance of 1½ inches (32 mm.) from the edge of the occiput. This usually placed the cephalic end of the tube at the lower border of the corpora quadrigemina.

At this time another “control” specimen was taken and the toxin introduced through the cannula. This was done slowly so as to avoid significant pressure changes. A total volume of 2 cc. of solution was used each time and the toxin was mixed with a few crystals of trypan blue in order to identify the area in which the toxin was placed. Trypan blue alone produces no systemic changes when so placed. The total amount of toxin used varied from 2.0 to 3.0 mg. in animals weighing 15 to 20 kg. There was usually some unmeasured seepage backward out of the fourth ventricle but the dependent position of the head resulted in flow of the material into the ventricular system. The opening into which the cannula was inserted was gently plugged with non-absorbent cotton and the wound packed with gauze. Venous blood specimens were taken for analysis at intervals of 15 to 30 minutes. The operations required 1 to 2 hours and the experiments were allowed to go on for 5 to 6 hours after the introduction of the toxin. They were terminated by the intravenous administration of 50 cc. of 10 per cent formalin and autopsy performed for tissue examination as well as to accurately localize the
PATHOGENESIS OF DYSENTERY INTOXICATION

cannula and area of exposure to the toxin as indicated by trypan blue staining of the ventricular walls.

RESULTS

Within a very short time after introduction of the toxin, the dogs developed a tachypnoea characterized by rapid, shallow respirations frequently associated with an expiratory grunt. Expiration was performed by a visible muscular effort, most noticeable in the abdominal musculature. This usually lasted for varying intervals up to 1 to 2 hours and was followed by what would be considered normal respiration. Along with these respiratory phenomena, there was a variable degree of tachycardia. At times more or less profuse salivation occurred and in three experiments diarrhea appeared, in one instance containing bloody mucus.

In several of the experiments autopsy showed that the catheter had not been correctly placed and we were able to trace its course, final location and outline the area of brain in contact with the toxin. In our earlier trials we found that the tube had occasionally not followed the iter but had perforated the cerebellar peduncles on right or left side and had ended in the subarachnoid space so that the trypan blue stained the cortex of the cerebrum and cerebellum. On one occasion the toxin was injected into the substance of the penduncles. In these cases the hematologic and other changes did not occur.

In a “typical” experiment in which the tube had been well placed, the dye outlined the walls of the third ventricle and the blue stain extended through the foramen of Monroe with faint coloration of the lateral ventricles. There was also some staining of the floor of the fourth ventricle and for a variable extent down the central canal of the

Fig. 1. Sagittal section of dog’s brain. 3-3, wall of third ventricle stained with trypan blue. L, margin of lateral ventricle similarly stained.
cord. The stain extended to a depth of 2 to 3 mm. into the walls of the third ventricle and massa intermedia and to a lesser extent elsewhere (Fig. 1). In such experiments there was a rapid rise in rectal temperatures shortly after administering the toxin. This was progressive and usually reached a peak of about 106°F, but on one occasion was 110°F. Along with this there was also a corresponding but less striking rise in hemoglobin content of the blood as well as hematocrit value. The latter tended to rise more than the former as though hypochromic cells were being released from some reservoir. The white blood cell count also rose but in a more striking fashion usually reaching a value almost double that at the start of the experiment. The rise was due to an increase in polymorphonuclear leucocytes with no striking changes in the percentage of the eosinophiles or lymphocytes. In several experiments there was a preliminary drop in the total leucocyte count to almost leucopenic levels followed by a rise about 2 hours after giving the toxin. The blood glucose content also showed a rise shortly after giving the toxin with marked variations in the degree of elevation in the different experiments. The rise, which on occasion almost doubled the control value, was, however, variable and fluctuated up and down during the experiments. The peak was usually reached about 1 to 2 hours after the toxin had been given and then gradually dropped to values close to the control level. There were no significant changes noted in the plasma chloride levels (Table I).

Occasionally trauma to the choroid plexus of the fourth ventricle resulted in some

### Table 1

**Experiment 8**

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Time after injection</th>
<th>Hematocrit</th>
<th>Hemoglobin</th>
<th>WBC</th>
<th>Sugar</th>
<th>Temperature</th>
<th>Remarks</th>
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<tr>
<td>1</td>
<td>0</td>
<td>44</td>
<td>88</td>
<td>10,300</td>
<td>111</td>
<td>101.4</td>
<td>Specimen taken; then nembutal 8 cc. i.v. 1:45 p.m.</td>
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<td>2</td>
<td>0</td>
<td>44</td>
<td>86</td>
<td>8,500</td>
<td>106</td>
<td>100.8</td>
<td>Operation completed 3:25 p.m.</td>
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<tr>
<td>3</td>
<td>0</td>
<td>44</td>
<td>84</td>
<td>9,200</td>
<td>109</td>
<td>101.0</td>
<td>Tube inserted 3:35 p.m.; toxin given 3:45 p.m.</td>
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<tr>
<td>4</td>
<td>4/4</td>
<td>46</td>
<td>90</td>
<td>7,500</td>
<td>114</td>
<td>101.8</td>
<td>Tachypnea</td>
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<tr>
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<td>4/4</td>
<td>47</td>
<td>88</td>
<td>8,100</td>
<td>122</td>
<td>101.8</td>
<td>Retching</td>
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<tr>
<td>6</td>
<td>4/4</td>
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<td>7,400</td>
<td>131</td>
<td>102.0</td>
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</tr>
<tr>
<td>7</td>
<td>1</td>
<td>47</td>
<td>90</td>
<td>6,800</td>
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<tr>
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<tr>
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<td>186</td>
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<tr>
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<td>4/4</td>
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<td>7,100</td>
<td>181</td>
<td>104.2</td>
<td></td>
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<tr>
<td>15</td>
<td>4/4</td>
<td>56</td>
<td>98</td>
<td>10,600</td>
<td>181</td>
<td>104.8</td>
<td></td>
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<tr>
<td>16</td>
<td>5/4</td>
<td>56</td>
<td>98</td>
<td>11,900</td>
<td>185</td>
<td>105.2</td>
<td>Experiment terminated</td>
</tr>
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bleeding in this area but this did not occur as a rule and multiple longitudinal sections of the area of the third and fourth ventricles and iter have shown intact ependymal lining in these locations.

In successfully conducted experiments the pituitary showed a very sharp demarcation of anterior and posterior lobes caused by intense congestion in the former. Histologically this congestion was occasionally accompanied by hemorrhage into the substance of this lobe.

While no abnormalities were noted in the esophagus, the stomach usually was intensely congested in the corpus and fundus; this involved the mucosa only and was often accompanied by petechial hemorrhages. On one occasion the latter were noted in the serosa as well. The antrum was usually not altered. Beginning at the pylorus the duodenum showed intense congestion reaching a maximum near the entrance of the bile ducts and then gradually lessening until the jejunum appeared normal. Occasionally the terminal ileum showed congestion and petechiae but this was not as intense as in the duodenum. In the colon the middle third usually showed more intense congestion, petechiae, and even gross hemorrhage into the lumen. The proximal and distal colon usually showed minor or no changes.

More variable structural changes were observed in other organs. The adrenals often showed congestion at the cortico-medullary junction. The pancreas occasionally showed small petechial hemorrhages in its substance. On two occasions there was edema of the gall bladder wall, most marked in its bed. The liver was quite "dry" on two occasions but usually was congested. The spleen was most often contracted, firm and "dry." The thymus showed focal hemorrhage in one case and was usually congested. There was no pulmonary edema though the larger pulmonary vessels were well filled. No clotting or thrombus formation was noted in any of the organs. The histological phenomena were similar to those previously reported.

DISCUSSION

Underlying the assumption that the central nervous system plays an important role in the pathogenesis of the visceral lesions due to Shiga toxin lies the hypothesis that these lesions result from a disturbance in the integrative mechanisms which regulate homeostasis. The complex of physiological and biochemical phenomena involved in this process keep the organism in a state of dynamic equilibrium within rather narrow limits, suitable for optimum function. When one or more components of this complex are activated by stimuli to significantly exceed this "normal" limit, abnormalities may appear in the homeostatic adaptation. From this point of view tissues have both a "private" and a "public" function (13). The former is essential to a maintenance of the integrity of the tissue structure; i.e., the local provision of tissue nourishment via the blood supply. To this end tissues have a variable degree of con-
control over their blood supply by means of local concentration of CO₂, histamine, etc. The “public” function is centrally controlled and integrated to serve the needs of the total organism. While the resultant shifts in blood distribution do not reach the level of tissue deprivation under ordinary circumstances, we hypothesize that in the presence of sufficient demand, such deprivation of the “private” blood supply may occur with resultant tissue injury. Under such circumstances the reactions on the part of the host to a parasite “may be so biologically disadvantageous that they contribute more to the pathology of the infection than any inherently aggressive mechanism of the invader” (14).

We view the arterio-venous anastomotic system in the various organs as the hydraulic mechanism under both local and integrated central control which regulates the distribution of blood in the processes involved in vascular homeostasis. This was already known to Claude Bernard in 1858 (15), who had observed that under certain circumstances venous pressure rose strikingly with arterial pulsations in the renal and portal veins which also contained more than normally arterialized blood. This mechanism served to maintain the blood supply to the heart and brain at the expense of the kidney and intestinal tract.

In our previous studies the attempt was made to disturb the functioning of the animals to a minimal degree compatible with the experimental purpose. To accomplish this we avoided any direct manipulation of the central nervous system. We felt that the toxin, transported to the central nervous system in some chemical complex conditioned by the transport function of the blood, would be released and act in an appropriate area. The latter, based on permeability properties, would most likely be located in the area of the floor of the third and fourth ventricles; i.e., close to the integrative vegetative centers of the hypothalamus and medulla. The tissues involved in the mechanism of the integration processes would be minimally disturbed by the experimental procedure and would presumably be activated in their usual manner.

In our present experiments, however, the toxin was brought into direct contact with the lining of the ventricles. There is evidence to indicate that absorption of substances from the cerebral ventricles differs significantly from those entering through the vascular circulation (16). Even though we were able to demonstrate an anatomically intact ependyma in the involved areas, there is no reason to believe that permeability remained unaltered under the experimental conditions. Furthermore, we have no way of evaluating the degree to which the normal mode of activation of the homeostatic mechanisms was influenced by this method of introducing the toxin. This latter could conceivably cause alterations in the order of appearance or intensity of the phenomena which we used as indices of activation of homeostasis, dependent on local factors as yet unknown. Our main purpose was to introduce the toxin into contact with the parenchyma of the central nervous system in a concentration at a
level below that, which on intravenous administration would produce a lesion. This would exclude such effects as might be caused by the transfer of the toxin from the ventricles into the systemic circulation. The latter effect would naturally also be diminished by the loss of toxin into the gauze packings of the wound due to backward flow of the injected material from the widely open cisterna magna.

The production of characteristic alterations of blood concentration, white blood cell count, hemogram count, and blood sugar under these conditions gives added support to the previous data indicating that they are somehow mediated through the central nervous system. In addition, the appearance of structural tissue changes under these circumstances adds further evidence to support the hypothesis that central integrative, homeostatic mechanisms may alter tissue blood supply to the extent of causing visceral lesions.

There are significant differences, however, between the data presented here, and those derived from the experiments in which the toxin was introduced intravenously in the otherwise intact animal. The regularity and order of appearance of the various phenomena showed more variation in these experiments and in general the magnitude of the changes was less. The variations in the sequence of appearance of the alterations may very well be due to the mode of administration of the toxin with variation in local absorption. We feel that the lessened intensity of the reaction is the result of the small amount of the toxin used. This, if it had been entirely absorbed, amounts to 0.1 μg/gm. The effective value is somewhat below this owing to the losses by drainage from the cisterna which was permitted in order to avoid the introduction of the effects of pressure increases in the ventricular system.

**SUMMARY**

The introduction of Shiga toxin into the ventricular system of the brain with major location in the third ventricle resulted in a response similar to that following the administration of the toxin either intravenously or by cross-circulation. The intravenous administration at the dosage level employed would have elicited no response. These observations lend support to the hypothesis that Shiga toxin activates some mechanisms in the central nervous system which are capable of producing visceral lesions. These mechanisms are those which control the vasomotor components of homeostasis. This hypothesis permits an explanation of the proximo-distal and intramural features of the lesion.

**BIBLIOGRAPHY**