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A TOXIC FACTOR IN EXPERIMENTAL TRAUMATIC SHOCK*

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MEDICAL investigations are often the product of a number of workers. This is particularly true in these years of war emergency, when groups of men co-operate in an attempt to solve problems quickly. Thus, in our search for a toxic factor in the shock syndrome, credit for the results is largely due to my associates, who have contributed much of the work and many of the ideas. Those who have worked throughout have been Drs. Austin Brues, Ira Nathanson, Alfred Pope and Paul Zamecnik and Miss Abby Nutt; others have collaborated in part of the work, namely, Drs. Waldo Cohn, René Dubos, Cynthia Pierce and Seymour Kety and Miss Dorothy Tibbetts. It is in their name that I make this summarizing report.

The history of the study of traumatic shock need only go back to World War I, when a group of illustrious physiologists studied the mechanisms involved in its production. This brilliant work was well summarized in their reports[1, 2] and in the book by Cannon.[3] Many of the physiologic responses that constitute the syndrome of traumatic shock were then delineated, and their cause was ascribed to an assumed but unisolated toxin. Subsequent attempts to isolate the toxin failed.

The period that lay between the two world wars was marked by a reaction against the toxic theory because of convincing studies, largely by Blalock[4] and Phemister,[5] which showed the predominant role of local fluid loss in the areas of trauma. And so we entered this conflict in the wise belief that the treatment for shock is transfusion, and readily available plasma has certainly saved many lives. Nothing I may say in this discussion should be construed in any way as detracting from this brilliant page of medical and, indeed, social progress.

I need not describe to this audience the clinical picture of traumatic shock. It is the delayed collapse that follows lacerating or crushing wounds, characterized by the well-known drop in blood pressure and diminution of blood volume.

With the onset of this war we again set ourselves to evaluate the possibility of toxic causative factors in shock. But why should one study the causes of shock? Surgeons say that there are many causes of this syndrome, but that point of view may change with further knowledge. It is true that the syndrome may develop from anesthesia alone, or that it may appear in relatively mild trauma or may be lacking in larger wounds. The reasons for this have not been clear, which merely proves that we have not yet learned its causes. Although shock may develop under diverse circumstances, true precipitating factors are probably not numerous. Depletion of the blood volume by hemorrhage will produce shock through obvious mechanisms, but shock — the so-called "toxic shock" — can also apparently arise from causes and through mechanisms that are less well understood. It is as true of this syndrome as of any disease that thorough understanding of its mechanisms is essential to logical therapy. Anyone who has observed the effects of bleeding and of trauma in animals or man is alert to the different toxic appearances of the traumatized animal. Something other than the large plasma loss appears to be involved. But this factor has appeared elusive. Moon[6] thought that he had found it by the implanting of autolyzed minced muscle tissue in the peritoneum. Scudder[7] had the ingenious idea that it was due to liberated potassium from traumatized tissues, though we now think that blood potassium rises only late in the shock picture.

The amount of work that has been done in this country on traumatic shock during this war has been large, and our knowledge of the subject is being well advanced. Much of this work is still unpublished and restricted, so that this discussion will be largely limited to recent publications and to our own unpublished data.

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Laboratory studies of traumatic shock have been characterized by the large number of divergent technics and divergent anesthetics in its production. This makes comparison rather difficult. All our observations have been made on dogs with Nembutal given intravenously in amounts sufficient to produce light anesthesia. We have used a new method to produce shock in animals. This was devised as a minimal insult so as not to produce overwhelming shock but to approach the shock state in a more quantitative manner than could be done by technics other than hemorrhage. The method developed by one of our associates, Dr. Nathanson, involves putting tight tourniquets around the two triceps surae, which include the gastrocnemius and soleus muscles. On release of the ties, after five hours, the blood supply becomes re-established and then these muscles swell enormously. The amount of edema that accumulates can be quite accurately estimated by a method, developed by Dr. Kety, of measuring the volume of the limbs.

But fall of blood volume and that of pressure are not the only manifestations of shock that deserve attention. In any experiment it is crucial that other physiologic observations also be made. For blood pressure may be misleading — it may be maintained for a surprisingly long time through vascular constriction, while the shock state may be developing, and then the pressure may fall rapidly, followed promptly by death. Other methods appear essential to judge the early progress of this syndrome — methods that indicate the state of the circulation. Progressive hemic concentration is one of the best technics, as it indicates the actual amount of plasma lost from the circulation. To evaluate the degree of shock we have used technics that originated in the work of one of us (J. C. A.[8-10]) in the last war. It was then shown that when shock is developing the oxygen content of the venous blood is much reduced, that the cardiac output falls, and also that the basal metabolism is reduced owing to the lack of adequate available oxygen. These reactions can all be explained on the basis of a slowing of the blood flow. Our judgment of the degree of shock, therefore, has been based on a fall of cardiac output and blood pressure, a rise in the resistance of the peripheral circulation, mainly that of the arterioles, and a decrease of blood volume. In early experiments we did not obtain all this information, but it has been accumulated in the main body of our recent studies. By means of these technics we were satisfied that we could approach the problem with a quantitative method for producing a minimal shock-producing trauma that would not be so overwhelming as to make study and treatment difficult. We also were able to measure fluid loss in this area progressively, as well as to estimate the beginning and progress of the shock state.

It soon became clear that it would be interesting to study the large amount of fluid that exuded from the anoxic muscles. A technic for this was arranged by enclosing the muscle in a thin rubber sheath from which the fluid could be collected by simple drainage. After the release of the tourniquet and the re-establishment of blood flow through the muscles, fluid fairly poured out into these investing sheaths. These simple operations could all be done with aseptic precautions, and after our first preliminary experiments, the usual surgical aseptic technics have always been employed.

Results

In the first problem we investigated we sought to find whether the shock state produced a changed permeability of body cells remote from the area of trauma, that is, whether the cells of organs such as the intestines and the liver allowed electrolytes and proteins to escape more freely than in a normal state. This was an arduous task undertaken by Dr. Brues, in which Dr. Cohn and Miss Tibbetts helped. These data are ready for publication and need no long discussion here. The conclusion was that there was no indication of any consistent shift of intracellular or extracellular water or electrolytes into or out of cells in shock except what occurred locally in the traumatized regions. In the traumatized areas there was evidence that the permeability of cells was considerably altered, so that extracellular salts were allowed to enter and intracellular salts were abnormally lost. Our experiments, however, have shown other characteristics of fluid exchange that appear interesting and important. At autopsy after prolonged shock one finds the intestinal mucosa full of blood, and Dr. Brues has observed that this appears to be markedly increased by transfusions of plasma or blood, showing that transfused fluids may be lost into this area. This indicates that the permeability of these capillaries has been altered by the shock state and that they therefore lose an abnormal amount of fluid.[11]

It also appears obvious that an abnormal circulation of the body fluids may occur about the area of trauma. Thus, a considerable loss of fluid may exude from the edematous traumatized muscles, sometimes amounting to as much as 200 cc. in five hours. If this fluid is not withdrawn but stays in the legs, it migrates away from the traumatized areas and some of it must be reabsorbed into the circulation. An accessory circulation of fluid is thus established, diffusing through traumatized tissues and being reabsorbed into the general circulation. This may affect the organism as a whole. This circulation, as well as that directly carried in blood vessels and lymph, easily explains the possible widespread effects of the local accumulation of toxic products from trauma. We have established absorption from the anoxic muscle mass by means of radioactive salts. These appear in the blood stream promptly after liberation of the muscle tie.

We have not done this to establish the absorption of the free fluid in the wound, but it could easily be done.

The minimal trauma that I have described produced traumatic shock in about a third of the animals. We then measured the fluid that collected locally from this operation together with the blood that was taken for our various analyses, and compared the incidence of shock in the groups that were subjected to the muscle ties with that obtained in control animals that were bled. There was little difference between the two series. The average indicated that we obtained a somewhat higher percentage of shock in the traumatized animal than in the hemorrhagic controls, but the total loss of blood in the animals that were traumatized was also slightly higher than that of the control animals. From these observations it appeared that the conception of Blalock[4] and Phemister[5] was correct and that the shock of trauma was nothing but a large local loss of fluid. But we were not satisfied with this conclusion because of the somnolence, lethargy and lack of sensibility to pain seen in patients who have suffered trauma, whereas patients who have had simple hemorrhage usually do not exhibit these signs so dramatically.

It appeared desirable to investigate the possibility that edema fluids contained some factor toxic to the organism. We therefore collected the fluid that exuded from the muscles that had been made anoxic. This fluid was easily collected aseptically by our technic, although the amount varied markedly in different animals. By using large animals from which to collect this fluid we could often obtain about 200 cc. from one dog. This slightly hemolyzed fluid could then be put into a small test dog to see its effect on blood pressure, cardiac output, peripheral resistance and hemic concentration. By this technic one was increasing blood volume, not decreasing it. If there were toxic, shock-producing factors in this fluid, it had to compete with this increased volume. Interestingly enough, nine out of thirty-two such fluids produced typical traumatic shock in recipient dogs. The others, however, had practically no effect following injection. Drs. Kety and Pope quite wisely inferred from these observations that the toxic factor in these fluids was not constant, and that it therefore probably arose not from the damaged muscle but rather from some extraneous source. This appeared to confirm the old toxic theory of the last war described in Cannon's book[3] and the more recent observations of Moon.[6]

We were convinced of a toxic element that was sometimes present in the sheath fluid that exuded from anoxic muscle. We therefore attempted to purify this chemically and, for this purpose, collected more than 2000 cc. of this fluid from a series of 9 dogs. It was collected with sterile precautions in bottles cooled in ice and promptly frozen in carbon dioxide snow. These fluids were combined, and the mixture proved to be shock-producing to several dogs. It contained some red cells, the hematocrit being about 1 per cent, had a specific gravity of 1.018 and tended to form a thin clot. An analysis of the inorganic salt content of this fluid showed that it contained much intracellular muscle fluid as well as extracellular fluid, for its potassium content was high and its calcium content low. It also contained less protein and more fixed acid than does blood plasma. The toxic factor in this muscle exudate was found to be a large molecule, for it was not dialyzable and was precipitated in the fraction that contained the protein. In this pool of muscle exudate Dr. Zamecnik also found high concentrations of intracellular proteolytic enzymes, and these enzymes were all contained in the fraction of ammonium sulfate precipitate that contained the toxic factor. We therefore sought to find whether these intracellular enzymes might be the factor that produced shock, but subsequent observations proved to Dr. Zamecnik that this was not so, inasmuch as fresh muscle extract that contained the same enzymes in high concentrations did not produce shock. Laborious attempts at purification, therefore, advanced our knowledge only in so far as they showed that the toxic molecule of this accumulation of muscle exudate was in the protein moiety, possibly in the gamma globulins, that it was destroyed by heat, and that its effects were not due to certain enzymes liberated from the damaged muscle. They did not disclose the actual substance that was producing shock in approximately one quarter of the test animals.

In many of these experiments bacteriologic studies of our muscle exudates were made. It soon became clear that, in spite of strict surgical precautions, all the muscle exudates were infected, most of them with organisms of the gas-gangrene group (in muscle exudates from 19 animals, Clostridia were found in 13, or 70 per cent). Coliform bacilli were not infrequently present, and in a few experiments *Staphylococcus albus* was found. Bacterial contamination was studied quantitatively to obtain an idea of the number of organisms present. Isolated organisms were also studied by Drs. Dubos and Pierce to see whether they produced toxins. The Clostridia were cultured to determine their type, and Dr. Dubos's laboratory found them to be the Welch bacillus (*Clostridium perfringens*).

When the number of these organisms was compared with the toxicity of the fluids, it became clear that the three fluids that produced shock in the series were among those that had the highest concentrations of Clostridia. In order to test this finding we assured the presence of an ample infection by injecting a culture of Clostridia into the triceps surae muscles at the time of ligation. In a series of 3 animals we injected Clostridia that had been isolated in previous experiments. These

muscles produced large quantities of fluid in which the amount of organisms varied as the fluid collection progressed. When the resulting heavily infected muscle fluids were injected into recipient animals, they produced profound shock when only one third of the usual amount of fluid was given. Removal of most of the organisms in the injected fluid was accomplished by high-speed centrifugation. The toxic factor did not appear to be the organism, for the supernatant fluid was equally toxic, whereas injected organisms resulted only in producing fever. A single similar observation was described in a report by the British Medical Research Committee[12] during the last war, in which edema fluid from the injection of *Vibrion septique* produced prompt and fatal shock. We next sought an antidote. Polyvalent Clostridia antitoxin was given to a dog a few minutes before injecting an extremely toxic sheath fluid. No shock developed. Our observations indicated that an anoxic muscle was an excellent medium for the growth of Clostridia and for the formation of its toxins.

We became interested in ascertaining why our tissues were so frequently contaminated with Clostridia. A dog's skin was prepared by shaving and thorough scrubbing with soap and water, followed by ether and alcohol solution, and then painting with strong iodine solution. After this strenuous preparation Clostridia could still be found on the intact skin. When Dr. Nathanson used electrosurgical technics with the greatest precaution, Clostridia were still found in the muscles deep in the normal anesthetized dog's leg. We are not sure that they exist there normally, for there is a possibility that spores may be carried into the muscle, but it is clear that they are frequently present in muscles in any traumatizing procedure. Our evidence, as well as confirmatory evidence,[13] demonstrated that Clostridia are practically constantly present deep in the skin of dogs. The presence of Clostridia has been noted before in dog muscles by Roome and Wilson,[14] although they thought them to be nontoxic. It is possible that they do not produce toxins even though they are present in the normal muscle, and that in traumatized muscles they can multiply and produce their usual toxins only in these relatively anaerobic conditions. This discussion becomes only of academic interest in these observations. The important fact is that the organisms are frequently present in traumatized dog muscle, and whether they are normally present or a contaminant makes little difference in the interpretation of our results. Dogs are the animals usually used for the study of traumatic shock, and the contamination of traumatized muscle by these naturally occurring Clostridia appears to us to be significant and to explain our varying cases of shock from muscle juice.

We next turned to the study of purified Clostridia toxins. These were obtained through the kindness of Dr. Milan Logan, who furnished us with the toxins of *Cl. perfringens* and of *Cl. oedematiens*. Injected intramuscularly these toxins produce different reactions. The Welch bacillus (*Cl. perfringens*) produces a great deal of local edema, possibly because its toxin is a lecithinase and therefore has a destructive effect on cell membranes. With this accumulation of fluid in the muscles there develop all the manifestations of shock that we have been determining — a reduced cardiac output, increased peripheral vascular resistance, a fall of blood pressure, a reduced blood volume and its accompanying increased hematocrit, and a lowered venous pressure. All these manifestations may be explained by the local effect of the toxin. We have not yet established whether there is a more general toxic action. When given intravenously this toxin produces marked hemolysis, and although shock is produced, it is different in this regard from our other experiments. We have observed, however, that moderate hemolysis is a common manifestation of shock in dogs, thus confirming the observation of Coonse et al.[15]

Cl. oedematiens toxin does not produce edema promptly when given intramuscularly, and intravenously it results in gradual shock without hemolysis. Following the administration of fatal doses there is often no immediate fall of blood pressure. The cardiac output declines gradually to very low levels, with an increased peripheral vascular resistance. The blood pressure falls terminally. At death there is intense congestion of the duodenum and small intestine, the liver is congested, and the adrenal medulla may be hemorrhagic. These findings are analogous to our pathologic findings in shock, except for the hemorrhagic adrenal glands.

The mechanism is known by which the Welch bacillus produces some of its effects.[16, 17] Macfarlane and Knight[18] showed that the so-called "alpha toxin" is an enzyme — a lecithinase that breaks down lecithin into smaller molecules. Lecithin is a constitutent of cell surfaces, so that destructive effects on capillaries or muscle cells can be understood, as well as the hemolyzing effect on red cells. A change of cell surface usually means a change in cell permeability, and so it is possible to explain the large effusions in the muscles where Welch bacillus toxin is placed. A similar enzyme is present in only small amounts in *Cl. oedematiens* toxin.[19] But this is not the only enzyme, for there is also present another group of enzymes called "hyaluronidases" or mucinases, or the so-called "spreading factor," that hydrolyzes hyaluronic acid. This acid is found in connective tissue, and the enzymic effect of destroying it results in a great increase of diffusibility through tissues and therefore in the spread of extracellular fluid and bacteria. One may thus explain the great edema, not only its presence adjacent to the area of toxin injection but also its widespread diffusion.

Has this work any significance in traumatic shock in the hospital emergency ward or at the front? In a series of eighteen elective orthopedic operations by Dr. Carroll Larsen and others, no Clostridia were found in human muscles. We must therefore decide that any Clostridia present in wounds are introduced at the time of injury. War wounds have been shown to have more than 30 per cent contamination with Clostridia, frequently without the appearance of detectable gas.[20] Such Clostridia, introduced into tissues that have not harbored them before, might be expected to be particularly likely to form toxins. The slow onset of shock seen in some of the wounded agrees with the length of time necessary for the proliferation of bacteria and the formation of their toxins. The importance of such organisms in the shock of war wounds appears to be a problem in regions of combat most urgently needing evaluation.

Our experiments have shown again the great importance of a loss of plasma fluid as a cause of traumatic shock, but they also indicate the importance of infection as a toxic factor. Clostridia that lurk normally in the dog multiply rapidly and produce their toxins in the excellent surroundings of traumatized anoxic muscles. The effects there will certainly accentuate the local fluid loss and so give a summation of effects. The toxin of *Cl. oedematiens* in the blood stream produces a shocklike state, but the mechanism is not yet clear to us. The time relations are satisfactory for allowing organisms to grow and for the onset of delayed traumatic shock. We think that the evidence is quite convincing that infection is the so-called "toxic factor" in traumatic shock in dogs.

We do not know as yet how widespread are the effects of the toxin. In our observations in the shocked animal it is clear that elevation of blood volume by transfusion is followed by markedly accentuated local fluid loss in the upper intestine and liver and lungs, as well as in the area of trauma. Such changes in permeability may be due to simple anoxia or to circulating bacterial toxins. This is a question we are in the process of trying to answer.

There may be other ways to poison the capillaries and venules in the way found characteristic of shock. It is not surprising that long anesthesia should be one such, and that the anoxia of hemorrhage should be another. We have searched hard for other toxins at the area of trauma, but we have not found them. In our experiments, fluid loss and infection appear to account for our incidence of shock. From a practical point of view it is good to have it so, for one can act constructively about them because methods for alleviating them both are available.

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