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ON THE OCCURRENCE, SITES AND MODES OF ORIGIN AND DESTRUCTION, OF PRINCIPLES AFFECTING THE COMPENSATORY VASCULAR MECHANISMS IN EXPERIMENTAL SHOCK[1,2]

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THE possibility was recognized by Cannon, Bayliss *et al.*[4] during World War I that positive deleterious principles might arise during hemorrhagic and traumatic shock, in consequence of the reduction in blood volume or tissue damage, and contribute to the fatal outcome. However, since little direct evidence has been forthcoming in support of this concept, there has been a growing tendency during recent years to emphasize the primary importance of the reduction in the effective blood volume and its direct circulatory consequences, to the exclusion of other factors.[5]

[1] The major portion of this material was presented at a conference on shock held under the auspices of The Josiah Macy, Jr. Foundation at Boston, May 14, 1945. Submitted for publication October 1, 1945.

[2] The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Cornell University Medical College. It was also aided by a grant from The Josiah Macy, Jr. Foundation to New York University. During the past summer, additional support and the facilities of the Lilly Research Laboratories, Woods Hole, Mass., were made available through Dr. G. H. A. Clowes, of the Eli Lilly Company.

[3] With the technical assistance of Mathilda Fischl and Leon Dziorney.

[4] W. B. Cannon, "Traumatic Shock," D. Appleton and Co., 1935.

[5] A. Blalock, "Principles of Surgical Care; Shock and Other Problems," C. V. Mosby Co., 1940.

Nevertheless, many phenomena observed in clinical and experimental shock appear to require a more adequate explanation, particularly those conditions leading to a state increasingly refractory to fluid replacement therapy; a condition which not only occurs in clinical shock, but which can be regularly induced in animals by certain standard experimental procedures. Nor can the fluid-loss concept explain the decreased tolerance to fluid loss and hypotension in traumatic as compared with hemorrhagic shock, or the death from tourniquet shock of animals whose limbs have been tightly taped to prevent significant fluid loss.

Attempts have been made, from time to time, to assign a definite role in shock to a variety of known agents derived from tissues, but none of these produce specific or systemic effects relating them directly to the changes observed in the conventional shock syndrome. Studies directed at the possible deleterious effects of metabolic derangements or tissue products resulting from reduced oxygen tensions in shock have revealed alterations in the chemical composition of the blood, which apparently reflect the relatively anaerobic character of metabolism during shock.[6] Thus far, however, except for the experimental data cited below, it has not been possible to causally relate any of these changes to the progressive development of irreversibility.

Recently, Chambers, Zweifach and their associates,[7,8] using the vascular reactions of the exteriorized omentum and mesentery as an index, have found that the shock syndrome in anesthetized animals subjected to prolonged hemorrhagic hypotension or to traumatic procedures consists of two stages, an initial compensatory and a subsequent decompensatory phase, referable to humoral factors with opposite actions. The initial phase was characterized by an increase in the frequency and amplitude of the intermittent constrictor activity (vasomotion) of the metarterioles and precapillary sphincters and in their heightened reactivity to epinephrine. The maintenance of this type of hyper-reactivity was favorable to the compensatory vascular adjustments to reduced blood volume, since, by confining the capillary blood flow to main thoroughfares, it served to insure an adequate venous return from the tissues. With the prolongation of the profound hypotensive state, hyper-reactivity was gradually superseded by a state of hypo-reactivity, characterized by a progressive depression in the reactivity of the terminal arterioles and precapillaries, antagonistic to the compensatory vasomotor adjustments which had prevailed during the initial stage of the syndrome. As a result, the peripheral blood flow was no longer confined to main vascular channels, but through loss of precapillary sphincteric control overflowed into the capillary bed; this, in turn, led to progressive venular stagnation and eventual failure of the peripheral venous return to the heart. Once this hyporeactive phase had fully developed, replacement therapy had only a transient effect; a state of irreversibility or failure to respond to transfusion having been reached. That humoral factors were responsible in large measure for both vaso-excitor and vaso-depressor effects was evident from their passive transference to anesthetized normal rats, where they produced corresponding effects on the vessels of the meso-appendix.

The development and persistence of a humoral vaso-depressor principle of this type in shock would appear to furnish a logical explanation of irreversibility. Its vascular effects are essentially decompensatory in nature. By interfering with the peripheral vaso-constriction essential for the maintenance of an adequate blood pressure and setting up conditions permitting the pooling of blood in capillaries and venules, it initiates a morbid cycle leading to a progressive decrease in the effective circulating blood volume. Once the vaso-depressor factor is dominant, transfusions could be expected to exert only a brief check on this morbid cycle and then to become lost from the effective circulation in the manner described above.

This novel and important contribution to the theory of shock provided the stimulus for the present studies. The experiments were designed to explore this concept further and, specifically, to investigate the sites of origin of these vaso-excitor and vaso-depressor principles as well as the mechanisms leading to their formation and destruction. The reasoning which guided our experimental approach may be briefly set forth. Since these principles were demonstrably circulating in the blood stream, their origin should be sought in the tissues of the shocked animal, from which they might be extracted under appropriate conditions. The sequence of their appearance in the blood should reflect the order of their production in the tissues. In considering the possible mechanism which might be responsible for their genesis, the most attractive, because most consistent, was the reduced oxygen tensions prevailing in the tissues during shock. This concept would be verified if the exposure of normal tissues to anaerobiosis *in vitro* resulted in the formation of similar principles. Finally, the ability of tissues to destroy these factors and the conditions under which destruction took place could be explored by similar *in vitro* experiments.

The utilization of the rat meso-appendix technique

[6] F. L. Engel, *Jour. Mt. Sinai Hospital*, 12: 152, 1945.
[7] B. W. Zweifach, R. E. Lee, C. Hyman and R. Chambers, *Ann. Surg.*, 120: 232, 1944.
[8] B. W. Zweifach, R. G. Abell, R. Chambers and G. H. A. Clowes, *Surg., Gyn. and Obstet.*, 80: 593, 1945.

of Zweifach and Chambers[9] for the detection of vaso-excitor or vaso-depressor activity in blood or tissue extractives provided a unique means for effecting a direct correlation between *in vivo* and *in vitro* phenomena. An evaluation of the nature and extent of such activity was made by following the changes in the responsiveness of the terminal vascular vessels to the topical application of epinephrine.

Intensity was graded by noting the concentration of epinephrine just sufficient to elicit an unmistakable constriction of the metarterioles in the meso-appendix after intravenous injection of the test samples and comparing it with that which had been required to effect a similar vaso-constriction in the control state before injection. *Duration* was measured by noting the time required for the vessels to regain their original reactivity. In keeping with the general character of this presentation, it has seemed adequate to express the effects observed as absent, slight, moderate or marked; and the abbreviations, VDM and VEM, will usually be used for vaso-depressor and vaso-excitor material respectively. The remainder of the technical procedures were those conventionally employed in the study of tissue metabolism and can most conveniently be described in connection with the experimental data.

Sites of Origin of the Vaso-depressor and Vaso-excitor Principles

The purpose of these experiments was to trace these humoral principles back to the tissues from which they were derived. Dogs in hemorrhagic and tourniquet shock and rabbits in tourniquet shock provided the material for these studies. Except when specifically noted, the dogs and rabbits were maintained under sodium pentobarbital anesthesia. Blood pressures were recorded from the femoral artery in order to insure fulfilment of the criteria for induction of irreversible shock. Blood samples were tested at frequent intervals for VEM and VDM, and at appropriate times during both compensatory and decompensatory phases a variety of tissues were removed for the preparation of saline washes. Thin slices of liver, heart, kidney and spleen and thin sheets of intestinal smooth muscle were prepared immediately after removal and shaken for 2–7 minutes with 5 parts by volume of iced physiological saline. Skeletal muscle was prepared for extraction by careful dissection of individual muscle fibers from the hind limbs just prior to sacrifice. Saline washes were cleared of cellular debris by centrifugation and used, as was serum, in 0.5 cc amounts for the rat meso-appendix test.

Since the major concern of these studies lay in the further exploration of the relation of the vaso-depressor principle to the condition of irreversible shock, emphasis will be given to the data bearing on this question.

The first group of experiments was designed to provide an answer to the question as to whether the production of VDM was a property common to all tissues or restricted to specific organ systems. To this end, tissues were removed for analysis from dogs and rabbits in the hypo-reactive, decompensatory stage of tourniquet and hemorrhagic shock, when VDM could be demonstrated in the blood stream. Saline washes of spleen, cardiac and smooth muscle were uniformly devoid of activity; those from kidney exhibited a profound vaso-excitor effect. Liver and skeletal muscle, however, invariably contained significant amounts of VDM, similar in its action on the vascular bed of the meso-appendix to VDM in serum from shocked animals. The concentration of VDM was always greater in liver washes made by shaking one part of tissue with five parts of saline than in serum simultaneously removed from the same animal, and similarly diluted with five parts of saline. An appreciable amount of VDM was found in skeletal muscle during hemorrhagic shock. This amount was considerably less than that found in the livers of such animals. Much larger amounts of VDM were found in the muscle in tourniquet shock. When skeletal muscle fibers were dissected out *in situ* before release of a five-hour hind-limb tourniquet, saline washes contained considerable amounts of VDM. Likewise, skeletal muscle washes obtained during the hypo-reactive stage two to four hours after release of the tourniquets contained approximately the same amounts of VDM as did the liver washes at this stage. Control studies on saline washes of tissues derived from normal animals were essentially negative.

It seemed reasonable to infer from these experiments that the VDM present in the blood during the hypo-reactive stage could be derived only from liver and skeletal muscle. In order to further relate the production of VDM to irreversibility, a second group of experiments was carried out to determine to what extent its production was confined to the hypo-reactive stage. Saline washes were made of a comparable series of tissues removed in the initial compensatory phase of shock, when only VEM could be detected in the circulation. No VDM was found in liver washes either in hemorrhagic or tourniquet shock. Skeletal muscle washes from dogs in this phase of hemorrhagic hypotension contained a small amount of VDM, which varied with the duration of the limb ischemia. Tourniqueted muscles likewise contained appreciable amounts of VDM. Kidney washes

[9] R. Chambers, B. W. Zweifach, B. H. Lowenstein and R. E. Lee, *Proc. Soc. Exp. Biol. and Med.*, 56: 127, 1944.

always elicited marked vaso-excitor responses. The remaining tissues were either neutral or contained scant quantities of VEM, probably due to blood contained in the organ. To determine whether the absence of VDM from the liver during the initial hyper-reactive stage might merely be due to the relatively short interval between the initiation of shock and the removal of the tissues, hemorrhagic shock was induced in unanesthetized animals. Such animals, if not treated with blood or plasma, persist in a hyper-reactive stage with only VEM in the blood until exitus and invariably respond favorably to transfusion. Livers removed from these hemorrhagic animals after as much as three hours of profound hypotension were likewise devoid of VDM.

The notable difference between the compensatory and decompensatory phases of shock with respect to the formation of VDM resides in the finding that no appreciable amount of VDM appears to be produced by the liver until after the onset of the hypo-reactive or irreversible stage. Two important implications of this observation may be pointed out. It suggests that the transition to the irreversible state is coincidental with and/or conditioned by the initiation of VDM production by the liver; and that the major portion of humoral VDM present during the hypo-reactive stage is derived from the liver. It is not possible at the present time to reconcile the presence of VDM in the limbs, particularly in tourniquet shock, during the hyper-reactive stage, with its absence from the circulation. It may be released in amounts too small to detect, because of the reduced blood flow in the extremities, or masked by the preponderance of humoral VEM; or, as will be shown later, it may be destroyed by the liver as rapidly as it is released into the circulation.

Before leaving this aspect of the study, brief consideration should be given to the relation of the kidney to the hyper-reactive phase of shock. Since the action exerted on the terminal vascular bed by VEM, present during this phase, is compensatory in character, its exclusive formation in the kidney would represent a major contribution by that organ to the maintenance of an effective circulation in the face of a reduced blood volume. Further evidence of the dependence on the kidney for the largest fraction of humoral VEM was provided by experiments on dogs after complete renal occlusion. They went into profound shock more rapidly than normal animals. Those in hemorrhagic shock tolerated less blood loss, and had only minimal amounts of VEM in the blood stream. VEM was entirely absent in those in tourniquet shock, and large amounts of VDM appeared with unusual rapidity. Apparently, the kidneys contribute not all, but certainly the major amount, of VEM. Other possible sources of similar excitor materials, particularly the pituitary and adrenals, are under investigation.

Mode of Origin of Vaso-depressor and Vaso-excitor Principles in Shock

Although experiments with saline extracts of tissues obtained from shocked animals served to localize the sites from which these principles were derived, they could provide only inferential evidence as to their mode of origin. The reduced oxygen transport to the extremities and abdominal viscera in shock seemed to offer the most likely explanation for their genesis; yet it was obviously impossible to exclude the participation of other, as yet unknown, factors. These considerations led to the utilization of *in vitro* methods to test the hypothesis that these agents might arise from metabolic derangements resulting from tissue anoxia, *per se*.

Accordingly, the same group of tissues taken from normal dogs and rabbits were prepared for *in vitro* studies in the manner described above and exposed to complete anaerobiosis for varying lengths of time, at 37.5° C. They were kept in either Krebs-bicarbonate medium or serum at pH 7.4, in an atmosphere of 95 per cent. N_2–5 per cent. CO_2. The usual period of exposure was 2 hours. The supernatants were then cleared of cellular debris and tested for VDM and VEM. Control experiments were carried out simultaneously in an atmosphere of 95 per cent. O_2–5 per cent. CO_2.

The *in vitro* results were in complete agreement with those with tissue extracts from shocked animals. Supernatants from spleen, cardiac and smooth muscle were entirely negative. Kidney uniformly produced VEM both in Krebs solution and in serum. Liver and skeletal muscle invariably produced VDM, similar in action on the vascular bed to VDM appearing in blood during the hypo-reactive phase of shock and in extracts of liver and skeletal muscle removed from shocked animals. The amounts formed during anaerobiosis were of the same order of magnitude as those present in washes of shock tissues. The rate of formation was much greater in liver than in skeletal muscle. With liver no VDM was detectible for about 20–30 minutes; thereafter the amounts increased quite rapidly and progressively throughout a three-hour exposure. Larger amounts appeared after incubation with serum than in Krebs-bicarbonate. Apparently complete anaerobiosis was not essential, since VDM was also formed by liver *in vitro* at oxygen tensions of 5 and 10 per cent., conditions more comparable to those prevailing in the liver during the hypo-reactive stage of shock. Cellular integrity was apparently necessary for maximal production of VDM, since only trivial amounts were formed by liver

brei under similar *in vitro* conditions. With skeletal muscle, relatively small amounts, comparable to those present in muscle extracts from animals in hemorrhagic shock, appeared after a two-hour anaerobic exposure; after five hours, the larger amounts formed corresponded to those in extracts from limbs tourniqueted for the same length of time. No VDM resulted from aerobic incubation of any of these tissues. Bacteriological studies, for which we are indebted to Dr. René Dubos,[10] excluded, in his opinion, the bacterial origin of VDM formed under these conditions.

In view of such complete agreement between the *in vivo* and *in vitro* experiments, tissue anoxia, *per se*, could supply an adequate explanation for the production of VDM and VEM in shock. The manner in which certain anesthetic agents influence the formation of these principles is under investigation. Reasons must also be found for the fact that VEM is elaborated by the kidney very early in shock, whereas the formation of significant amounts of VDM by the liver has been observed only in the hypo-reactive stage. Since anoxia leads to the formation of both principles, it may be concluded that, despite the reduced blood flow to the liver during the hyper-reactive phase, enough oxygen is transported to support an oxidative type of metabolism; whereas the reduction in oxygen supply to the kidney is sufficient to initiate anaerobic processes. A number of observations favor this conjecture. The oxygen requirements of the kidney are much higher than those of the liver; and it has been shown by *in vitro* studies that the respiration of the kidney is much more sensitive to reductions in oxygen tension than that of the liver. The blood supply ordinarily is considerably in excess of the respiratory requirements of the liver; its oxidative metabolism remains unaffected when the hepatic artery is made the sole source of supply.[11] Our own experiments indicate that the oxygen needs of the liver are met during the hyper-reactive phase, since, as will be pointed out, the respiration of liver slices obtained in that stage proceeds at a normal or even somewhat higher than normal rate. The kidney, on the other hand, has been shown by Van Slyke and Phillips[12] to suffer a profound reduction in blood flow from the very onset of the shock syndrome. Furthermore, the formation of VEM by the kidney *in vitro* proceeds very rapidly under anaerobic conditions. Additional evidence of the trigger character of the production of VEM is provided by our observation that, even after removal from normal animals, significant amounts of VEM have usually formed during the brief period of anoxia between the removal of the kidney and the preparation of the extract.

[10] Personal communication, 1945.

[11] F. L. Engel, H. C. Harrison and C. N. H. Long, *Jour. Exp. Med.*, 79: 9, 1944.

[12] Personal communication, 1944.

Mode of Destruction of Vaso-depressor and Vaso-excitor Principles

Once significant amounts of VDM have appeared in shock, this principle persists in the circulation until death, subject to only temporary dilution by transfusions. On the other hand, VDM injected into the normal test rat disappears from the circulation usually within 10–20 minutes, depending on its initial concentration. Does the persistence of VDM in the shocked animal result from a loss of a protective function possessed by the normal animal? Should this be the case, might not this loss constitute a critical defect and ultimately be responsible for the failure to respond to transfusions? The ineffectiveness of the temporary restoration of aerobiosis by massive transfusions in the hypo-reactive stage of shock suggests that the mechanism for the destruction of VDM has already suffered profound damage.

The *in vitro* approach appears to have provided answers to these questions. Tissues from normal animals were incubated aerobically at 37.5° C. with VDM obtained from various sources. Spleen, kidney, cardiac, smooth and skeletal muscle proved incapable of destroying VDM. On the other hand, normal liver slices invariably destroyed VDM from whatever source obtained, in the course of a 2–3 hour aerobic incubation. This held true for VDM in serum and saline washes of liver or skeletal muscle from shocked animals, as well as that formed *in vitro* by anaerobic incubation of normal liver and skeletal muscle. Destruction of VDM was thus found to be restricted to the liver.

In sharp contrast with healthy liver slices, those from animals in hypo-reactive shock, as well as normal liver slices previously exposed to anaerobiosis for two hours, destroyed only small amounts, or, usually, none at all; and occasionally even contributed additional VDM despite the aerobic conditions of the experiments. Anaerobiosis thus had two undesirable consequences for the liver; it led not only to the formation of VDM, but also to a concomitant loss of the capacity to destroy it, on subsequent restoration of aerobic conditions. An entirely different picture was presented by liver removed during the hyper-reactive stage; not only was VDM absent, but the capacity to destroy it was fully preserved.

An attempt was made to relate the respiratory metabolism of the liver to the capacity to destroy VDM. Normal liver slices which had lost this property as a result of previous anaerobic incubation were found to have sustained a profound reduction in oxygen consumption, which usually fell to 25 to 35 per cent. of the control values. Conversely, livers

removed during the hyper-reactive phase and which retained this function consumed oxygen at a normal rate. This apparent correlation between the over-all oxidative capacity and inactivation of VDM was, however, not borne out by similar studies with liver removed during the hypo-reactive stage. Here the total loss of capacity to destroy VDM was frequently associated with a reduction in oxygen consumption of as little as 15–20 per cent. below the average control values. Destruction of VDM would therefore appear to be a function of some specific, and probably enzymatic, system in the liver which is extremely sensitive to anoxia. Further support for this assumption is furnished by our success in preparing a cell-free extract from normal liver, by methods which will be described elsewhere, which has proved capable of destroying VDM on aerobic incubation *in vitro*.

The fate of VEM in the shocked animal was also explored by *in vitro* methods, in an attempt to provide an explanation for its gradual disappearance from the blood stream. Since its actions are such as to assist the compensatory vascular mechanisms by which the capillary bed is kept ischemic and the reduced blood volume confined within the main vascular channels, its loss puts the organism at a disadvantage in its efforts to maintain an effective circulation. One important mechanism not susceptible to *in vitro* analysis is the probable inaccessibility of kidney VEM to the blood stream as a result of the progressive and profound reduction in renal blood flow characteristic of the shock syndrome. Several other limiting factors have, however, been disclosed by the exposure of VEM to a variety of tissues *in vitro*. Liver and kidney slices inactivated VEM on aerobic incubation; kidney appeared to possess the additional property of inactivating VEM during the latter stages of prolonged anaerobiosis.

VEM production and destruction by kidney under anaerobic conditions may be described in some detail in view of their possible relation to the disappearance of vascular hyper-reactivity. When the kidney is exposed to anaerobiosis *in vitro,* the formation of VEM takes place with great rapidity, and reaches a maximum in about an hour; thereafter production gradually diminishes and ceases altogether after the second hour, even in the presence of freshly replaced serum substrate. With the further prolongation of anaerobiosis, the VEM previously formed gradually disappears. Presumptive evidence of the anaerobic destruction of VEM *in vivo* has also been obtained. Kidneys removed from animals in the hyper-reactive, as well as in the hypo-reactive, stage unprolonged by large transfusions invariably contained significant amounts of VEM. When, however, dogs were maintained in the hypo-reactive stage of hemorrhagic shock for long periods by means of repeated small transfusions, the kidney contained minimal amounts of VEM or none at all. These kidneys, on subsequent anaerobic incubation *in vitro,* no longer produced VEM. Thus, at least four factors were found to contribute to the disappearance of VEM from the circulation—aerobic destruction by the liver and kidney, limited accessibility to the circulation due to reduced renal flow, limited anaerobic production by the kidney, and eventual disappearance with prolongation of renal ischemia and anoxia.

It might be appropriate at this time to inquire into the significance of the disappearance of VEM from the circulation for the development of the hypo-reactive or irreversible stage of shock. Apparently VEM is needed for the maximal compensatory response of the peripheral vascular system. Its absence or excessive destruction in the presence of a reduced blood volume should hasten the onset of a hypo-reactive state by making it impossible for the animal to restrict the peripheral circulation sufficiently to insure an adequate venous return. As a result, a morbid cycle would be initiated through the further pooling of blood and the further reduction in circulating blood volume and blood pressure. At some time in this morbid cycle, oxygen transport to the liver should be reduced to the point where anaerobic processes would be initiated leading to the production of VDM and the development of the hypo-reactive phase.

The Relation of the Vaso-depressor Principle to the Development of Resistance to Shock

The method of inducing traumatic shock in rats by exposure to the Noble-Collip drum provides a convenient means for studying factors contributing to increased susceptibility or resistance. Under standard conditions, the mortality rate is directly related to the number of revolutions, and resistance to drum trauma can be regularly induced by repeated sublethal exposures. Our studies were concerned with the possibility that mechanisms associated with VDM formation and destruction might be involved in the development of increased susceptibility and resistance to this type of shock.

It was first necessary to determine whether VDM was produced in shock in rats, as well as in dogs and rabbits. This was found to be the case. Following graded exposures to the Noble-Collip drum, saline washes of livers removed immediately or 30 minutes after drumming yielded VDM in amounts proportional to the number of revolutions and expected mortality. The effects of this VDM on vascular reactivity were indistinguishable from those observed with VDM obtained from dogs and rabbits. Under *in vitro* conditions, normal rat liver also duplicated

the anaerobic production and aerobic destruction of VDM, previously observed with normal livers from dogs and rabbits.

For the study of the relation of VDM production to resistance, 3 sets of rats were used: normal rats on a Purina Chow diet; rats on a low protein (5 per cent. casein) diet for 10–12 days in order to increase their susceptibility to shock[13] and rats on Purina Chow, made resistant to 1,000 revolutions by repeated sublethal exposures to drumming.[14]

The following order of susceptibility to drum shock was obtained. Rats on a low protein diet were highly susceptible, over 80 per cent. mortality occurring with exposure to as few as 300 revolutions as compared with normal rats where a similar mortality rate was not reached until the animals were exposed to 650 revolutions. Trauma-resistant rats showed only a 10–15 per cent. mortality following drumming for 650 revolutions.

The next question investigated was whether, on anaerobic incubation, liver VDM formation might proceed at different rates in these three groups of rats, relative to their varying susceptibility to shock. The interesting result was obtained that, in the course of a 2-hour anaerobic incubation, livers from the resistant group produced less VDM than the normal controls or low-protein group.

Even more significant were the observations that not only the formation, but also the inactivation of VDM by the liver was related to resistance and susceptibility to shock. Thus, liver slices from resistant rats, anaerobically incubated for two hours, retained their capacity to destroy VDM when returned to aerobic conditions in contrast to liver slices from normal controls and low-protein rats which uniformly lost their ability to destroy VDM under similar conditions. This resistance of the inactivation system to the usual destructive effects of anaerobiosis is, so far, unique; its nature remains to be investigated. The *in vitro* findings on resistant rats were reinforced by experiments in which VDM formation and destruction by liver were determined after 1,000 revolutions in the Noble-Collip drum. Not only was much less VDM present after 1,000 revolutions than in livers of normal rats given much less drumming (600 revolutions), but the livers of resistant animals retained the capacity to destroy, on aerobic incubation, the VDM which had already been formed.

A comparison was also made of the respiratory behavior of livers from these 3 groups. In contrast with the usual reduction in oxygen consumption of shocked livers from non-resistant rats, those from resistant rats after drumming exhibited a somewhat higher than normal respiratory rate. In both this respect and in the retention of the capacity to inactivate VDM, livers of resistant rats after drumming behaved like livers removed from dogs in the hyperreactive phase of shock. Much less difference was observed in the capacity of the livers from these different groups to resist the depressant effect of a previous 2-hour anaerobic incubation on the subsequent rate of oxygen consumption. After 1 hour of anaerobiosis, the respiration of livers from resistant rats was slightly, and possibly significantly, higher than that of the normal or low-protein group; this slight superiority was lost after 2 hours of anoxia. The retention of the inactivating capacity by livers of resistant rats after anaerobic incubation despite the marked reduction in respiration offers additional evidence that the inactivation of VDM is not a function of the over-all oxidative capacity of the liver, but is related to some specific process, presumably of an enzymatic character.

A further observation relevant to the association of VDM to susceptibility to shock was derived from the study of the reactivity of the terminal vascular bed of the meso-appendix of rats given the low-protein diet. The initial reactivity remained normal through the 10th day of the diet; by the 12th day, however, initial reactivity to epinephrine was depressed and the response to injected VDM was more pronounced and prolonged than in the normal controls. Thus, even without trauma, a state of diminished vasomotion and depression of response to epinephrine had already set in, and the capacity of the liver to inactivate VDM was apparently already reduced. It was of additional interest that the livers of these animals, initially pale and yellowish, rapidly became red and hyperemic following the injection of VDM, suggesting a pooling in that organ; the kidneys were also moderately congested. Similar changes have not been observed in normal rats. This engorgement of the liver following the injection of VDM is reminiscent of the congestion in that organ during the hyporeactive stage of shock.

The concordant results of a variety of experimental approaches suggest that, at least in this type of shock, resistance and susceptibility are linked to factors which not only influence the rate of VDM formation in the liver but also augment or diminish the resistance to anoxia of the mechanisms in the liver which inactivate or destroy VDM. The exact manner in which these variations are induced by training and diet should present a rich field for investigation. The possible participation of VEM in the development of resistance and increased susceptibility to shock is being further investigated to round out the picture. Preliminary experiments with rats have indicated

[13] G. Toby and R. L. Noble, personal communication, 1945.

[14] G. Toby and R. L. Noble, *Can. Jour. Med. Res.*, 22: 79, 1944.

that a prolonged low protein régime leads to a reduction in the capacity of the kidney to produce VEM.

THE VASO-DEPRESSOR PRINCIPLE AS A "TOXIC" FACTOR IN IRREVERSIBLE SHOCK; OTHER IMPLICATIONS

We are now in a position to review briefly the evidence supporting a causal relationship between the vaso-depressor principle and irreversible shock:

(*a*) The temporal association of humoral VDM with vascular hypo-reactivity and failure to respond to transfusions has been previously established by Zweifach, Chambers and associates.

(*b*) The origin of VDM has now been traced to the liver and skeletal muscle; its genesis, to tissue anoxia. The time relations of its production in these tissues to its appearance in the circulation indicate that in hemorrhagic shock the liver is the major source of VDM; in traumatic shock, the damaged skeletal muscle mass may provide an additional amount of VDM sufficient to account for the differences between these two types of shock.

(*c*) The effects of VDM are such as to interfere with the compensatory vascular mechanisms necessary to maintain an adequate circulation in the face of a reduced blood volume. Its persistence in the blood stream would set up a morbid cycle leading to a progressive and ultimately fatal reduction in effective circulation.

(*d*) Transfusions have been shown to effect only a temporary reduction in the concentration of VDM in the blood; thereafter, the morbid cycle is re-established and leads to the pooling of the transfused blood.

(*e*) The aerobic destruction of VDM by healthy liver provides a mechanism for liberating the vascular bed from the deleterious effects of VDM.

(*f*) This function is impaired in the liver of animals which have passed into the hypo-reactive phase of shock. The same environmental condition, namely, tissue anoxia, which leads to the formation of VDM also results in progressive damage to the system which can destroy it.[15]

(*g*) In one type of shock (Noble-Collip drum), variations in susceptibility have been directly related to variations in the formation of, and in the capacity to inactivate, VDM. Resistance was associated with the retention by the liver of the capacity to destroy VDM after drumming or after anaerobic incubation.

[15] Further evidence that the liver plays an important role in the development of irreversibility to transfusion is provided by vivi-perfusion experiments of Frank, Seligman and Fine. Dogs subjected to prolonged hemorrhagic hypotension were vivi-perfused from the carotid artery of a donor through the jugular or splenic vein for a period of 2–3 hours. It was possible to prevent the development of irreversibility by vivi-perfusion of the liver, whereas controls perfused through the jugular vein died soon after transfusion. (Personal communication.)

It may be asked whether VDM constitutes the only toxic factor which can be specifically related to the development of irreversibility. A number of blood[16] and tissue extractives have, from time to time, also been suggested as specific toxic agents in shock. These include histamine, potassium, callicrein,[17] adenylic acid, adenosine triphosphate,[18] acetylcholine and bacterial toxins, particularly those formed by Clostridia[19] under anaerobic conditions prevailing in damaged muscle. Although many are capable of producing hypotension and a variety of vascular effects and several are lethal in high concentrations, all were found to differ in at least one essential respect from VDM; none lowered the reactivity of the metarterioles and precapillaries to epinephrine. Since reduced responsiveness to epinephrine is an invariable characteristic of the hypo-reactive stage in the conventional shock syndrome, it would seem justifiable to require of a suspected toxic factor that it reproduce the decompensatory vascular phenomena which are consistently present during this phase of shock. This does not preclude the possibility that some of these agents may, under special circumstances or in non-specific fashion, contribute to the lethal outcome, just as increased blood viscosity resulting from hemoconcentration, while not essential for the development of irreversibility, will intensify the decompensatory effects of VDM.

Several questions have arisen in our minds with respect to terminology. The terms "vaso-depressor" and "vaso-excitor" have been selected for these principles as descriptive of their property of either depressing or enhancing the reactivity of the terminal vascular bed to epinephrine, as well as influencing in a similar manner the vasomotion of the metarterioles and precapillary sphincters. We suspect that these effects are achieved indirectly, by the potentiation or inhibition of the fundamental reactions which govern the behavior of the smooth musculature of these blood vessels. While the term "toxic" could legitimately be applied to the end results of the action of the vaso-depressor principle in shock, it would be an inappropriate designation for the principle itself unless it could be shown to constitute a pathological product of metabolism peculiar to shock. Observations of a preliminary character have indicated that this is not the case and that the vaso-depressor material will prove to be a physiological principle, detrimental to

[16] H. N. Green and H. B. Stoner, *Jour. Physiol.*, 103: P30, 1944.

[17] W. W. Westerfeld, J. R. Weisiger, B. G. Ferris, Jr. and A. B. Hastings, *Am. Jour. Physiol.*, 142: 519, 1944.

[18] M. Bielschowsky and H. N. Green, *Nature*, 153: 524, 1944.

[19] J. C. Aub, A. M. Brues, R. Dubos, S. S. Kety, I. T. Nathanson, A. Pope and P. C. Zamecnik, *War Med.*, 5: 71, 1944.

the compensatory vascular mechanism in shock only as a consequence of the excessive concentrations reached.

These observations, as well as a consideration of the specific effects of the VEM and VDM on the terminal vascular bed, have led us to entertain the hypothesis *that the vaso-excitor and vaso-depressor principles are oppositely acting components of a homeostatic mechanism participating in the regulation of peripheral blood flow and blood pressure.* The renal vaso-excitor is very probably similar to or identical with the renin-angiotonin system, to judge from their action on the terminal vascular bed. The hypertensive effects of angiotonin have been securely established; what has hitherto been lacking is its physiological hypotensive counterpart. The vaso-depressor principle of hepatic origin fulfils, with respect to its action on the terminal vascular bed, the requirements of an antagonist to the renal vaso-excitor principle. The former depresses, the latter enhances the vasomotion and epinephrine reactivity of these blood vessels. The former increases the relative duration of the dilator phase of vasomotion in the metarterioles and precapillaries, thereby reducing peripheral resistance; the latter prolongs the constrictor phase and increases peripheral resistance. By virtue of these vascular effects, a shift in equilibrium towards the predominance of the hepatic vaso-depressor would favor the development of hypotension, while the preponderance of the renal vaso-excitor would predispose towards a hypertensive state. The competition between these two principles for the control of the terminal vascular bed is seen during the progression of the shock syndrome, particularly in the transition from the hyper- to the hypo-reactive phase.[8] During this transitional stage, both principles are present in the blood stream, but their concentrations are such as to balance one another, thus permitting the vascular bed to briefly resume a normal type of reactivity. While their concentrations during this phase of shock are undoubtedly much higher than under normal conditions, this may represent the type of equilibrium prevailing at normal blood pressure levels in the healthy organism. Should such prove to be the role of this new liver function in the economy of the organism, we would suggest the name "hypotensin" for the hepatic vaso-depressor we have described. The validity of this vascular homeostatic concept is now under investigation in a variety of experimental and clinical conditions.

THERAPEUTIC IMPLICATIONS

What are the therapeutic implications of these studies for the management of experimental shock which has become unresponsive to transfusion? At least two requirements of a successful therapeutic régime, not met by fluid replacement alone, have been revealed. The immediate goal consists in the liberation of the vascular bed from the decompensatory influence of VDM; the ultimate, in the arrest and reversal of the progressive dysfunction of the liver with respect to VDM formation and inactivation, which results from prolonged anoxia. Once these ends were attained, transfusions would be rendered capable of restoring an effective circulation and increasing peripheral resistance sufficiently to maintain an adequate blood pressure. With these requirements in mind, certain procedures have suggested themselves as a basis for further investigation.

The immediate problem of freeing the vascular bed from the deleterious influence of VDM can be approached in several ways. It might be possible to antagonize its actions by supplying VEM in amounts sufficient to insure the resumption of the compensatory type of vascular reactivity, which in turn would lead to the restoration of an adequate blood flow to the tissues, particularly to liver and kidney. The re-establishment of aerobic conditions in the liver should, to judge from *in vitro* experiments, halt any further production of VDM, thereby limiting the problem to the counteraction or inactivation of the VDM which had been previously released into the circulation. In addition to direct antagonism by VEM, the possibility should also be considered of inactivating VDM in the blood stream by appropriate agents. Mention has already been made of a cell-free liver extract which has proved capable of inactivating VDM under aerobic conditions *in vitro*.

The success of any of these procedures would ultimately depend on whether or not they eventually led to the reversal of the liver disability with respect to VDM formation and inactivation. *In vitro* studies have shown that the restoration of aerobiosis for as long as 3 hours to liver slices from shocked animals, is unsuccessful in repairing the damage to the inactivating mechanism. These experiments are supported by the results obtained by Frank, Seligman and Fine[20] with animals in irreversible shock, given infusions containing angiotonin for relatively short periods of time (2–3 hours); the beneficial effects of angiotonin on blood pressure and cardiac output did not persist much beyond the period of administration. The possibility is not excluded that spontaneous repair of the liver defect might eventually take place, were these measures sufficiently prolonged. However, in addition to the restoration and maintenance of aerobic conditions, the liver might still require support in the form of specific liver enzymes or substrates, to enable it to recover from the damage sustained. Some or all of these agents appear to be

[20] Personal communication, 1944.

present in the liver extracts mentioned above, to judge from their *in vitro* inactivation of VDM.

One further therapeutic possibility may also be profitably explored. The importance of the waning humoral concentration of VEM for the development of hypo-reactivity has already been emphasized. It was also pointed out that the capacity of the kidney to form VEM was definitely limited, not only under anaerobic conditions *in vitro,* but also in shocked animals maintained for long periods in the hypo-reactive phase. Consideration should therefore be given to measures which might enhance the endogenous formation of VEM by kidney and thus permit the spontaneous restoration of a state of equilibrium between VEM and VDM, by which the decompensatory vascular effects of the latter would be abolished.

It is evident that the investigation of these potential therapeutic procedures, as well as the vascular homeostatic concept which has been advanced, will be greatly accelerated by the elucidation of the chemical nature of VEM and VDM as well as by the isolation of the enzyme systems and substrates concerned with their production and inactivation. We wish to make clear that our present experimental results and the inferences we have drawn from them pertain only to hemorrhagic and traumatic shock in animals; and that it would be premature to extend them, at this time, to the condition of shock in man.

OBITUARY

EDWARD WILBER BERRY

February 10, 1875–September 20, 1945

THE death on September 20, 1945, of Edward Wilber Berry brought to a close one of the most unusual careers in American science, that of a man with little formal education who became a notable figure in university life and a leader in the science of geology.

Born on February 10, 1875, at Newark, N. J., he was educated in the local schools, completing his formal education with the high school in 1890. Shortly afterward he became a cotton goods salesman, remaining in this profession for seven years. He then went into newspaper work, spending the eight years from 1897 to 1905 as president, treasurer and manager of the Passaic (N. J.) *Daily News.* He had had from boyhood a bent toward rocks and fossils that developed while he was still a journalist into a keen amateur talent for paleobotany. The period from 1902 to 1905 was marked in his scientific career by a series of papers on the paleobotany of New Jersey, some descriptive, some philosophical. This work attracted the attention of Professor W. B. Clark, head of the Department of Geology at the Johns Hopkins University and State Geologist of Maryland, who in 1905 brought Berry to Baltimore to help prepare reports on the Cretaceous deposits of Maryland. In 1907 Berry was appointed assistant in paleobotany at the Johns Hopkins University, progressing from this beginning to become professor of paleontology in 1917, dean of the faculty of philosophy in 1929, and provost of the university in 1935. He had been appointed geologist with the U. S. Geological Survey in 1910 and assistant state geologist of Maryland in 1917. He retained all these connections until his retirement in 1942.

He married Mary Willard in 1898 and found in her a faithful consort, whose sudden death in 1939 was a severe blow to him. Two sons survive, Professor E. Willard Berry, of Duke University, and Dr. Charles T. Berry, of Stonington, Connecticut.

Berry's scientific work earned him honors from many organizations. He was president of the Paleontological Society in 1924. He was president of the Geological Society of America at the time of his death. He was a fellow of the American Academy of Arts and Sciences, American Association for the Advancement of Science, American Society of Naturalists. He was a member of the National Academy of Sciences, the American Philosophical Society, the Washington Academy of Sciences, the Torrey Botanical Club, Société géologique de France, Academia nacional de ciencias en Córdoba (Argentina), Sociedad geológica del Perú. He was awarded the Walker Prize of the Boston Society of Natural History in 1901, the Mary Clark Thompson Medal of the National Academy in 1944. Lehigh University gave him an honorary doctorate of science in 1930.

The scope of Berry's work was wide, and his volume of production stupendous—the total product of his activities is some 500 articles, ranging from short notes to extensive treatises. He began with the paleobotany of the Mesozoic deposits of the northern Atlantic Coastal Plain, expanding through his connections with the Federal Survey and some of the State Surveys to include in his descriptive studies ultimately the floras of the Mesozoic and Cenozoic deposits of the whole area of the Atlantic and Gulf Coastal Plains. Out of these descriptive studies came many discussions of phylogeny and other philosophical aspects of paleobotany. Occasionally he dealt with Paleozoic floras also. Collections of fossil plants from various parts of Latin America came early into his hands, turning his attention to those regions and leading him eventually to make several trips to South America, an extensive tour in the Andean region in