THE VASCULAR PROPERTIES OF TRAUMATIZED AND LAKED BLOODS AND OF BLOOD FROM TRAUMATIZED LIMBS*

By Dallas B. Phemister, M.D.

of Chicago, Ill.

If the circulation to a limb is obstructed its arterioles and capillaries dilate. On release of the obstruction, reactive hyperæmia follows, lasting about as long as the obstruction, during which time the limb is flushed and increased in volume and the arterial limb pressure is lowered. It occurs equally well whether the limb is innervated or denervated. What causes this vasodilatation? Anrep, Lewis and Grant have assumed that a vasodilator metabolite—histamine or a histamine-like substance—is formed in the asphyxiated tissues during the period of hyperæmia. This substance is either destroyed *in situ* or washed away in the venous blood. To test such blood for vasodilator action, use was made of Anrep's viviperfusion apparatus. This consists of a flask attached to a cannular system which can be inserted in the course of an artery. The flask can be shortcircuited by means of a T bone-tap, emptied and filled, and its contents then circulated by again adjusting the tap. The apparatus was inserted in the course of the femoral artery of a dog and permitted of recirculation of blood collected from the femoral vein during the period of hyperæmia after recovery of the limb from the effects of the obstruction. The dog was anæsthetized with ether and the limb was denervated to exclude nervous influences, so that the changes observed must be explained on a physical or chemical basis; 0.4 of a gram of heparin was injected to prevent coagulation of blood and the perfusion apparatus was inserted in the course of the femoral artery just below Poupart's ligament. For the collection of venous blood either a Y cannula or a second perfusion apparatus with flask was inserted in the course of the femoral vein. A plethysmograph was applied to the limb for measuring limb volume, a mercury manometer was connected with the cannula in the femoral artery and another with a cannula in the carotid artery. Limb volume, limb pressure and carotid pressure were recorded on a long paper kymograph. The results of certain experiments with this method were reported by Phemister and Handy (*The Journal of Physiology,* 1927, vol. lxiv, p. 155). It was found that the circulation of the nontraumatized (unshaken) blood collected from the femoral vein of the limb during the period of hyperæmia caused no vasodilatation; that is, if a vasodilator substance is formed in the tissues during the period of obstruction it is not washed out by the blood to an extent which makes its presence demonstrable by this method. Ordinary venous blood behaves likewise.

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If either arterial or venous blood flowing through the flask is shortcircuited for a period up to four or five minutes and the stagnant blood then sent through the limb (in case of venous blood the flask with its tubes clamped off must be transferred to the arterial side), it causes either no vascular changes or the changes are very slight. If either arterial or venous blood is withdrawn and shaken for a few seconds to a few minutes before insertion into the flask, it produces vasodilatation on circulation. If either arterial or venous blood is shortcircuited in a flask containing a bead, the inlet and outlet tubes clamped and the flask detached and shaken without exposure to either air or light, it produces vasodilatation. Arterial or venous blood, the gaseous content of which has been modified by exhaustion of gases and recharging with varying quantities of O_2 or CO_2, always caused vasodilatation. However, the blood was always traumatized in making the modification.

The longer the traumatism the greater the vasodilatation up to a certain point. The maximum effect from shaking is reached in two to three minutes, beyond which shaking for as long as ten minutes produces no increase.

What change comes about in the blood as a result of this amount of traumatism that gives it this vasodilator property? Have vasoconstrictor substances been destroyed, or has a vasodilator substance been liberated or created from blood elements? The two vasoconstrictor hormones, adrenalin and pituitrin, are far too stable from chemical standpoints to be destroyed by such slight traumatism as is necessary to give the reaction, and when blood has been traumatized to a point where it gives a maximum dilator action it is necessary to add at least five times as much adrenalin or pituitrin as is necessary to give vasoconstriction in order to obliterate this vasodilator effect.

Is a vasodilator substance liberated or created by traumatizing the blood elements? When blood is shaken for two to three minutes which is enough to produce a maximum vasodilator effect, the pH remains constant or practically so; consequently, the reaction is not the result of change in the acid-base balance.

Is histamine or a histamine-like vasodilator substance liberated from the blood cells by breaking them up? If so, the greater the amount of traumatism the greater the amount of such substance liberated and the greater should be the vasodilator effect. Laking, or hæmolysis of blood by freezing and thawing, breaks down nearly all of the cells and should most effectively liberate the vasodilator substance. Also, the greater the amount of traumatized blood circulated the greater should be the vasodilator effect. Consequently, quantitative tests were made by the circulation of different amounts of blood traumatized for varying lengths of time, of laked blood and of blood to which different amounts of histamine were added. When more than 75 c.c. were used, blood was added from another dog to keep blood volume up to normal. It was found that the greater the amount of histamine added to a given quantity of blood and the greater the quantity of histaminized blood circulated the more marked the vasodilator effect. With large amounts of histaminized blood entering the animal there was an initial increase in limb volume, fall

in limb pressure and then fall in general blood-pressure. But after one to one and a half minutes both limb and general vascular beds would acquire the power of resisting much of the histamine action, although when 500 c.c. of blood were used the histamine would continue to enter the circulation in much the same concentration for three and a half to four minutes.

The quantitative tests with traumatized blood were made as follows: Blood was traumatized by shaking for a few seconds to one hour and the effects were determined of circulating amounts varying from 30 to 475 c.c., as previously stated. When 30 c.c. were used the maximum vasodilatation was produced by shaking for two to three minutes; prolongation of the act for ten or twelve minutes producing no alteration in the effect; but shaking for twenty minutes caused vasodilatation followed by vasoconstriction, and shaking for forty to sixty minutes caused vasoconstriction from the beginning, which lasted for three to five minutes, after which vascular response was very much diminished.

Increase in amount of slightly traumatized blood circulated did not increase proportionately the vasodilator effect. Thus 30 c.c. of blood shaken for two or three minutes cause vasodilatation, which reached its maximum in twenty to twenty-five seconds and disappeared in fifty to seventy seconds; 75 c.c. produced slightly more effect than did 30 c.c., but 220 or 475 c.c. produced no greater effect than did 75 c.c.; 475 c.c. of blood traumatized for two to five minutes caused a maximum dilatation in thirty to thirty-five seconds, which wore off in sixty to ninety seconds despite the fact that undiluted traumatized blood was still going through the limb; however, when 475 c.c. of blood shaken in 50-c.c. lots for one hour were circulated there was limb constriction lasting for eight minutes followed by slow recovery.

Thus blood that had been slightly traumatized caused vasodilatation lasting a short time, and increase in the quantity of blood circulated beyond approximately 75 c.c. did not increase the duration of the vasodilatation; blood that had been traumatized extensively, as by shaking for twenty minutes, caused vasodilatation followed by vasoconstriction, and blood traumatized very severely, as by shaking for one hour, caused vasoconstriction only. The greater the quantity of severely traumatized blood circulated, the longer the duration of the vasoconstriction.

Quantitative tests were made with laked blood, which was prepared by freezing with liquid oxygen and thawing. Amounts were circulated varying from 1 minim to 220 c.c. One to 10 minims injected into the tube caused vasodilatation, which increased with increase in the amount of blood injected; but 1 c.c. caused beginning limb dilatation followed by limb constriction and 10 c.c. or more caused limb constriction only, and the greater the amount the longer the constriction lasted.

When untraumatized blood is treated with very small amounts of laked blood it produces vasodilatation on circulation, but as much as 5 to 10 per cent. produces beginning vasodilatation, followed by limb constriction and larger percentages cause limb constriction only.

Since blood traumatized in varying degrees acted similarly to blood that had been laked in varying degrees, it was thought probable that their action was produced by the same thing and that traumatism had produced hæmolysis. Consequently, blood that had been shaken for lengths of time varying from a few seconds to one hour was centrifuged and the plasma examined for the presence of hæmoglobin. It was found to be present in amounts that varied directly with the amount of traumatism, ranging roughly from 0.0573 gm. per 100 c.c. for blood shaken for thirty seconds to 3.689 gm. per 100 c.c., or over 20 per cent. of the total hæmoglobin for blood shaken for fifty-five minutes. Microscopic examination also showed evidences of breaking down of red cells. Consequently, the hæmolysis is responsible for the vasomotor properties of both traumatized and laked bloods.

The facts that small amounts of hæmolyzed blood or of traumatized blood caused vasodilatation and fall in regional blood-pressure when circulated through the limb raised the question as to their effect when injected into the general circulation. To determine this point, 475 c.c. of blood shaken for four or five minutes, or to which 5 to 10 c.c. of hæmolyzed blood were added, were circulated from a flask connected with the femoral vein. In none of several experiments was there any considerable amount of alteration in general arterial blood-pressure. It was thought possible that the immediate reactions from indirect blood transfusion in man might be due to the traumatism resulting from mixing with the sodium citrate solution; but the absence of any considerable fall in general blood-pressure when traumatized blood was inserted into the general circulation in dogs argues against that view.

Traumatic shock has been regarded by many investigators as due to a toxic substance derived from the traumatized tissues entering the general circulation and producing vasodilatation with resultant low blood-pressure. It might even be conjectured that a vasodilator substance is absorbed from the damaged extravasated blood in quantities sufficient to produce a state of shock.

A test for the presence of vasodilator substances in the blood from a traumatized limb was made in the following way: A viviperfusion apparatus was inserted into the femoral vein of a dog, a constrictor was applied distally and the limb was severely hammered. A second animal was prepared in the usual way with a viviperfusion apparatus in the femoral artery and a plethysmograph on the limb. Carotid blood-pressure tracing was then established for the first dog and the constrictor removed. The limb swelled rapidly afterward and the animal's blood-pressure gradually fell in the course of a few minutes to one hour to the level of 80 to 50 mm. of Hg., which is within the range of that ordinarily encountered in traumatic shock. The venous blood flowing through the flask was then shortcircuited, the connecting tubes clamped, the flask detached and connected with the artery of the second dog without traumatizing blood in the transfer. Circulation of such blood several times in three experiments usually caused very little or no vasodilatation; but if the blood was withdrawn from the flask and shaken for a short time it produced the usual vasodilator reaction. Therefore, if there is a vasodilator

substance in the blood coming from the traumatized limb of an animal which produces shock, it is not present in sufficient quantity to be detected by this method.

The experiment does not support the theory that shock is a traumatic toxemia from a histamine-like substance formed in the traumatized tissues and causing vasodilatation of the general capillary bed. Furthermore, necropsy examination of the traumatized limb showed increase in limb volume from hemorrhage which was sufficient to account for the fall in blood-pressure. In fact, the volume of blood that it was necessary to withdraw intermittently in the course of an hour in order to kill an animal was always less than the increase in volume of the traumatized limb of the other animal, which was due very largely to hemorrhage in the tissues.

The nature of the substance or substances liberated by traumatizing or laking the blood which produce the vascular changes here recorded have not been determined, but they appear to be the same in both cases. They are rendered active by the hæmolysis. The vasodilatation which results from a small amount of hæmolysis may be due to one factor, and the vasoconstriction which results from a large amount of hæmolysis may be due to another factor, or they may both be due to the same thing acting in different concentrations. The quantitative tests speak against the action being the result of histamine or a histamine-like substance.

No definite clinical phenomena can at present be explained by these reactions. But since such marked vasodilator properties can be created in the blood by mild injury and slight hæmolysis, it is not unreasonable to assume that these properties may be acquired in certain cases of injury or disease and that they may be responsible for certain unexplained circulatory symptoms.