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Hepato-Renal Factors in Circulatory Homeostasis.* II. Disappearance of Hepatic Vaso-Depressor Material Following Intravenous Administration.

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Recent *in vivo* and *in vitro* studies[1,2] have established the existence of a hitherto unrecognized vasodepressor principle which enters the blood stream in increasing amounts during the decompensatory stage of hemorrhagic and tourniquet shock. The vasodepressor principle, VDM, was found to be elaborated by the liver and skeletal muscle whenever the blood supply to those tissues was reduced below levels necessary to maintain an oxidative type of metabolism. The quantitative contribution of the liver is considerably greater than that of the skeletal muscle. Maximum blood levels are reached in experimental shock when the hypotension is profound and sufficiently prolonged to result in the development of a state unresponsive to transfusion, the so-called irreversible state.

Once the vasodepressor material appears during profound shock and precipitates the stage of vascular hypo-reactivity, it persists in the blood and is only temporarily diluted when the animal is transfused. The persistence of vasodepressor activity in the blood of shocked animals is in contrast to its transient effect when injected into the blood stream of normal rats for bio-assay. This points to the existence, under normal conditions, of mechanisms for removing this principle from the circulation. *In vitro* incubation experiments[2] have demonstrated that the healthy liver is the only tissue which possesses an enzyme system capable of oxidatively destroying VDM. However, its removal from the blood stream in the living animal may be a more complex phenomenon, with several accessory mechanisms potentially participating in clearing the blood of VDM. Among these are the possible renal excretion of VDM into the urine, the inactivation of VDM by some component of the blood, and the counterbalancing of the vasodepressor effect by the release from the kidney of the oppositely acting vasoexcitor principle, VEM.

A consideration of the mechanisms concerned with the precise regulation of VDM blood levels should weigh the relative contributions of both the liver and kidneys in clearing VDM from the circulation. Experiments were therefore carried out to determine the rate at which endogenous or exogenous VDM was cleared from the blood stream of rabbits with the liver and kidneys intact, and with either the liver or the kidneys, or both, excluded from the circulation.

Methods. The disappearance of *exogenous* VDM was followed by the assay of blood samples taken at intervals following the intravenous injection of a VDM concentrate prepared from anaerobic beef liver (method of preparation used by Dr. A. Mazur to be published elsewhere). The vasodepressor activity of the heparinized plasma samples was measured by the rat meso-appendix technic[3] which uses the reactivity of the terminal arterioles and precapillaries to epinephrine as an index of vasotropic effects. The potency of the liver concentrates was such the 0.1 γ

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1 Zweifach, B. W., Lee, R. E., Hyman, C., and Chambers, R., *Ann. Surg.*, 1944, **120**, 232.

2 Shorr, Ephraim, Zweifach, B. W., and Furchgott, R. F., *Science*, 1945, **102**, 489.

3 Chambers, R., Zweifach, B. W., Lowenstein, B. E., and Lee, R. E., Proc. Soc. Exp. Biol. and Med., 1944, **56**, 127.

to 0.5 γ of VDM (on the basis of nitrogen content) produced a 20- to 30-minute vasodepressor effect in normal, 125 g rats, an effect similar to that produced by the injection of 0.5 cc of plasma from dogs in irreversible hemorrhagic or tourniquet shock.

Four types of experimental animals were used:

(1) *Controls with liver and kidneys in the circulation.* Normal rabbits, and rabbits which had been partially eviscerated according to a technic described by Engel,[4] leaving intact the arterial blood supply to the liver via the hepatic artery. (2) *Arenal animals.* The renal artery and vein were tied off to exclude the kidneys from the circulation. (3) *Hepatectomized animals.* Complete evisceration was carried out so as to exclude the liver from the circulation but leave the kidneys intact. (4) *Hepatectomized-arenal animals.* Complete evisceration excluding both the liver and kidney from the circulation.

A series of 16 rabbits, anesthetized with Seconal (25 mg per kg) were used in this group of experiments. Following the operative procedures the rabbits were transfused with 20 to 40 cc of whole blood or 5% albumin until the blood pressure became stabilized in the 90 to 100 mm Hg range. Control blood samples gave a neutral bio-assay. Two to 3 cc of the liver VDM concentrate were then injected intravenously and samples of femoral vein blood (2 to 3 cc) were removed within one to 2 minutes and at 10-minute intervals thereafter, until one hour had elapsed. For assay, about 0.5 cc of plasma was injected intravenously into normal rats in which the meso-appendix had been exposed for microscopic observation. The amount of VDM present in the injected blood samples was determined by noting the length of time that the terminal arterioles of the test rat remained unresponsive to epinephrine. The details of this method of assay are given in previous publications of Chambers and Zweifach.[1,3]

The disappearance of *endogenous* VDM was studied in the partially eviscerated rabbit preparation in which the hepatic artery was left as the sole source of blood supply to the liver. Temporary hepatic anoxia was produced by placing a clamp on the hepatic artery for varying periods of time, for the purpose of inducing VDM formation in the liver. The re-establishment of the hepatic circulation after a 45- to 90-minute period of liver anoxia led to the appearance of VDM in the blood stream. The disappearance of endogenous VDM was followed in 5 rabbits with either both the liver and kidneys in the circulation, or with the kidneys excluded.

Results. A. *The Disappearance of Exogenous VDM.* 1. *Controls, Liver and Kidneys in Circulation.* The most rapid removal of VDM from the blood was found in the control series of animals in which both the liver and kidneys were intact. As shown in Fig. 1, blood taken one to 2 minutes after injection of vasodepressor concentrate gave a 25- to 35-minute VDM bio-assay and then rapidly fell off in activity, becoming neutral within 30 to 40 minutes. The higher the initial blood concentration of VDM, the longer was the period required for the animal to clear the blood of this principle.

2. *Arenal Animals.* When the kidneys were tied off and the liver allowed to remain in the circulation, the VDM blood levels showed a more gradual decline, the depressor principle remaining in the blood somewhat longer than in control animals. In Fig. 2 it can be seen that the VDM was not completely cleared from the blood until 40 to 50 minutes had elapsed. The gradual disappearance of VDM in these animals closely resembled the progressive inactivation of VDM which occurred when liver slices were incubated aerobically *in vitro* with similar vasodepressor solutions.[2]

3. *Hepatectomized Animals.* On the basis of *in vitro* inactivation experiments in which only the liver could inactivate VDM, it might be predicted that exclusion of the liver from the circulation should result in the accumulation of VDM in the blood. This, however, was not the case; in eviscerated-hepatectomized animals with an intact kidney circula-

[4] Engel, F. C., Harrison, H. C., and Long, C. N. H., *J. Exp. Med.*, 1944, **79**, 9.

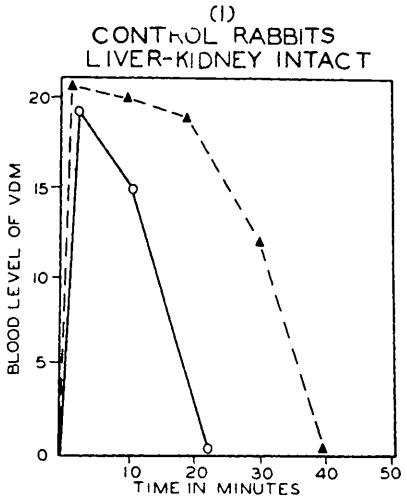

FIG. 1. *Control Rabbits*—includes a normal rabbit (○) and a partially eviscerated rabbit with the hepatic artery to the liver intact and with the kidneys in the circulation (▲).

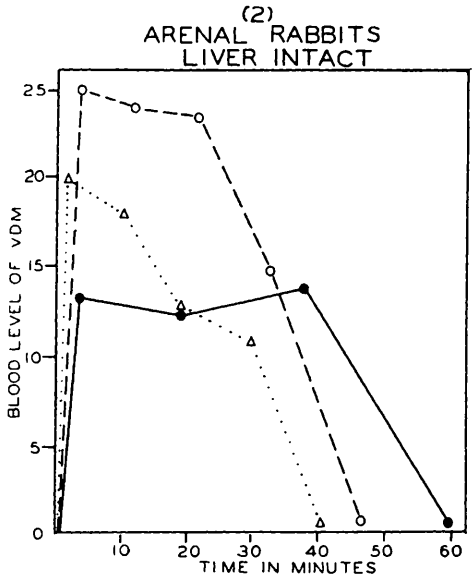

FIG. 2. *Arenal Rabbits*—includes 2 hepatic artery preparation animals (△, ○) and one normal animal (●).

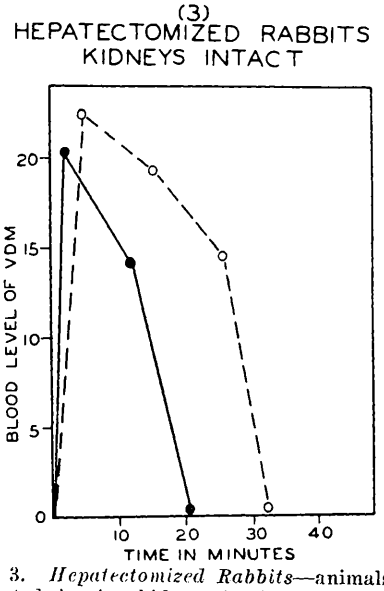

FIG. 3. *Hepatectomized Rabbits*—animals were eviscerated, leaving kidneys in circulation.

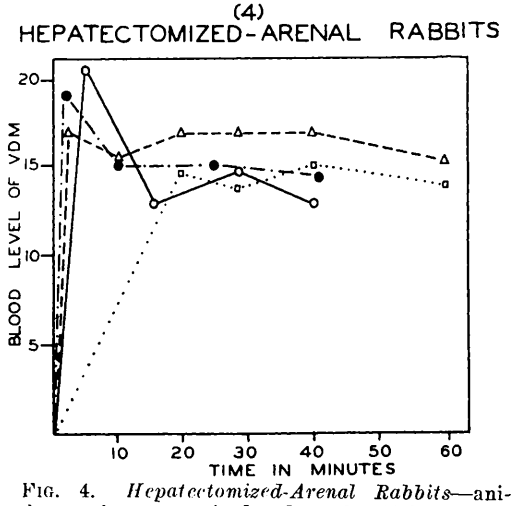

FIG. 4. *Hepatectomized-Arenal Rabbits*—animals were hepatectomized and renal pedicle tied off.

FIG. 1, 2, 3, 4.

Graphs of VDM blood levels following the intravenous injection of a vasodepressor concentrate prepared from anaerobic beef liver. The amounts of vasodepressor material in the blood were determined by the rat meso-appendix test and are expressed along the ordinate axis in terms of the number of minutes that the arterioles and precapillaries remained unresponsive to epinephrine.

tion, VDM disappeared from the blood within 30 to 40 minutes after its intravenous administration (Fig. 3). Since kidney tissue cannot inactivate VDM, another renal mechanism, presumably excretion, appeared to be the means for its removal under these conditions. This inference was confirmed by experiments on the dog in which urine samples were collected and tested for vasodepressor activity. A dog was hepatectomized and the kidneys left in the circulation. Blood and urine samples were taken following the

intravenous injection of the liver vasodepressor concentrate, the urine being collected from the bladder by means of an in-dwelling catheter. In order to eliminate the undesirable effects on the rat test of the concentrations of urea and electrolytes in the urine, it was dialyzed against distilled water overnight in the refrigerator before being tested. Considerable quantities of VDM were found in the urine after the intravenous injection of VDM, whereas the control urine showed only a mild vasodepressor activity. The excretion into human urine of a principle having similar vascular depressor effects has recently been demonstrated in this laboratory by Dr. R. F. Furchgott.

The difference between the controls and the arenal animals points to the participation of the kidney in the removal of VDM from the blood. Additional evidence for such participation was obtained from experiments in which meso-appendix assays were made on both normal rats and arenal rats using the same VDM sample. When the kidneys were tied off, the depressor effects of VDM on the peripheral vessels persisted for longer periods than in normal rats.

Blalock[5] and more recently Van Slyke and co-workers[6] have shown that renal blood flow is reduced to negligible levels in profound shock. The loss of the renal-excretory route for VDM disposal undoubtedly serves as an additional factor to perpetuate the blood vasodepressor activity and thereby contributes to the development of an irreversible state.

4. *Hepatectomized-Arenal Animals.* The kidneys and the liver appear to be the only tissues concerned with the removal of VDM from the blood. When both these organs were excluded from the circulation of the rabbit, the blood level of VDM remained significantly unaffected over a period of an hour, the duration of the experiment (Fig. 4).

The persistence of VDM activity in the blood in the absence of the liver and kidney indicated that the blood *per se* was not concerned with VDM inactivation. This was further confirmed by the observation that plasma or serum suffered little or no loss in VDM activity during aerobic incubation at 37.5°C for 2 to 3 hours.

Previous *in vivo* experiments[2] have demonstrated that the capacity of the liver to inactivate VDM is progressively impaired in shock by the hypoxic state in the organ. In the present series a similar situation was encountered in 3 rabbits which had developed profound hypotension for about 60 minutes following accidental blood-loss during the evisceration procedure. The rabbits were then transfused with large amounts of whole blood until they had returned to normal blood pressure levels. In one animal the kidneys were then tied off and the VDM concentrate injected into the blood stream. No significant removal of VDM occurred and the animal went into profound shock following the removal of only 8 cc of blood for testing purposes. In the other 2 rabbits the kidneys were left in the circulation. Despite the presence of the kidneys, the intravenous administration of VDM was likewise followed by its persistence in the circulation and by a progressive fall of blood pressure to shock levels.

B. *The Disappearance of Endogenous VDM.* These studies were made on a special preparation in which the gastro-intestinal tract was completely removed depriving the liver of its portal blood supply but leaving the hepatic artery intact. Long and co-workers[6] have found that the liver in such animals is maintained in a healthy condition for at least 4 to 5 hours, the duration of their observation. In our own studies, the aerobic state of the liver metabolism under these circumstances is indicated by the absence of VDM in the blood. When the hepatic artery was occluded for 60 minutes and then released, considerable amounts of VDM appeared in the blood stream within 15 minutes. In animals with an intact renal circulation, the VDM disappeared from the blood within 60 minutes after release of the hepatic clamp. When the kidneys were tied

[5] Blalock, A., and Levy, S. E., *Am. J. Physiol.*, 1937, **118**, 734.

[6] Van Slyke, D. D., and co-workers, *Conference on Hemorrhage, Ann. N. Y. Acad. Sci.*, 1946.

off before releasing the clamp on the hepatic artery, large amounts of VDM accumulated in the blood. The blood pressure fell to shock levels within 45 minutes. Evidently, the VDM inactivating mechanism of the liver had been damaged by the period of anaerobiosis and was no longer capable of inactivating the VDM in the blood.

Summary. *In vitro* studies have shown that the vasodepressor principle, which appears during the hypo-reactive stage of shock and results from the anoxia of liver and skeletal muscles, can be inactivated under aerobic conditions by healthy liver slices. The present study was concerned with the mechanisms by which endogenous and exogenous VDM are removed from the circulation of the living animal. The exogenous VDM was concentrated and purified from saline extracts of anaerobic beef liver. The endogenous VDM was released into the blood following liver anoxia produced by temporary occlusion of the hepatic artery in a partially eviscerated preparation. Two mechanisms for VDM removal were revealed: (1) its inactivation by the healthy liver; (2) its excretion into the urine by the normal kidney. A preliminary period of hepatic anoxia rendered the liver incapable of inactivating VDM *in vivo,* presumably through anoxic damage to the hepatic enzyme system for this function. This situation is analogous to the progressive impairment of the VDM inactivating mechanism in the liver which develops during the course of shock and which is considered responsible for the perpetuation of the hypo-reactive state of the peripheral vascular system. The loss of the renal excretory function for VDM during hypo-reactive shock deprives the animal of an important means of clearing the blood of VDM and thereby aiding in liberating the vascular bed from this decompensatory vasotropic principle.

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Functional Components of the Greater Superficial Petrosal Nerve.

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Larsell and Fenton[1] early emphasized the importance and pointed out the inadequacies of our knowledge of the greater superficial petrosal nerve. Later Chorobski and Penfield[2] added emphasis to the subject by demonstrating that the nerve is an important pathway for vasodilator fibers of the seventh cranial nerve to the pial arteries. More recently Foley and DuBois[3] experimentally separated the functional components of the nerve in a limited number of animals and described its varieties of sensory and motor fibers. Numerative studies on the functional components of the greater superficial petrosal nerve, in a satisfactory number of animals, are needed to complete our knowledge of the composition of the nerve.

Methods. The motor nerve fibers of the greater superficial petrosal nerve of the right side of 9 cats and one dog were eliminated by cutting the rootlets of the facial nerve within the cranium. This operation produced a nerve that, aside from an occasional easily identifiable[3] sympathetic fascicle, consisted only of sensory axons derived from the geniculate ganglion of the seventh cranial nerve.

After allowing a minimum of 14 days for disappearance of the motor axons, the right degenerated and left normal nerves of each

1 Larsell, O., and Fenton, R. A., *Laryngoscope,* 1928, **38**, 371.

2 Chorobski, J., and Penfield, W., *Arch. Neur. and Psychiat.,* 1932, **28**, 1257.

3 Foley, J. O., and DuBois, F. S., *J. Comp. Neur.,* 1943, **79**, 79.