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ABSTRACT

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1. Preparing Institution	New York University Medical Center
2. Title of Report	ETIOLOGY OF EXPERIMENTAL SHOCK.
3. Principal Investigators	(10) B.W. Zweifach, Ph.D., M. Wurzel, M.D. and A. Janoff, Ph.D.
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1. Lysosomes in Normal and Tolerant Animals Subjected to Shock. ~~Work~~ was continued to test the hypothesis that the release by cells of the hydrolases contained in cytoplasmic lysosome granules into the circulation may exacerbate tissue injury and contribute to the irreversible trend during shock. Adaptation or tolerance is associated with a greater stability of lysosome particles even under in vitro conditions. Mitochondrial systems were not similarly ~~stabilized~~. Agents which predispose to shock (Thorotrast) or which nullify tolerance appear to do so by a direct action of this colloid on the lysosomal membranes of Kupffer cells and other macrophages. The local release of lysosomal enzymes appears also to contribute to the genesis of local tissue injury, since depletion procedures (excess vitamin A) inhibit local inflammatory reactions as well as the local hemorrhagic necrosis induced by combinations of bacterial endotoxins and epinephrine.

2. ~~Vasoactive Factors Originating in Blood.~~ A new polypeptide, ~~(designated as~~ (SVPx, serum vasoactive peptide unknown), ~~has been~~ purified and concentrated from the blood plasma and serum of rabbits and man. ~~The substance is assayed by a modification of the isolated aortic strip method.~~ In high concentrations, it is as effective a contracting agent as norepinephrine. In low concentrations ~~(even below threshold levels for muscle contraction)~~, the material strongly potentiates the action of constrictors such as norepinephrine. SVPx has been found not to be any of the known synthetic polypeptides and is not identical with any of the other preparations of tissue or blood which have constrictor properties.

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(2) ETIOLOGY OF EXPERIMENTAL SHOCK. (17) VA (3)

The current report deals with two separate aspects of our program: (I) Alterations in the lysosomal enzymes during shock coincident with modifying procedures in the form of "tolerance" or "blockade" (in collaboration with Dr. A. Janoff); and (II) biochemical and pharmacologic studies of polypeptides in blood acting to increase smooth muscle tone (in collaboration with Dr. M. Wurzel).

I. LYSOSOMES IN SHOCK

As indicated previously (see 1962 report), liver lysosomes are disrupted following hemorrhage and trauma and their contained hydrolases released into the circulation (1). The presence of large amounts of free, highly active hydrolases may exacerbate tissue injury and contribute to the development of irreversibility during shock. Further work on animals adapted to stress indicated that tolerance is associated with an increased stability of hepatic lysosomal particles.

As a further extension of these studies on the mechanism of tolerance, observations were made under comparable circumstances on the membrane stability of another group of particles in the liver cells, the mitochondria. It was found that tolerance induced by repeated injections of E. coli endotoxin, as well as the enhanced resistance engendered by cortisone, were not associated with any stabilizing action on mitochondrial systems. The stabilization of lysosomal particles is therefore specific and not a generalized adaptive phenomenon.

The converse phenomenon, that of increased susceptibility, was shown, in the case of the nullifying effect of Thorotrast on the endotoxin tolerant state, to

be due to a direct action of this agent on lysosomes. Thus, Thorotrast appears to act directly on the integrity of lysosomal membranes of Kupffer cells and other macrophages to counteract the stabilizing influence of the adaptive procedure per se (2).

Evidence is also available that local release of lysosomal enzyme is involved in the tissue damaging action of bacterial endotoxins--particularly those lesions elicited by combinations of epinephrine and endotoxin. Procedures designed to deplete tissues of lysosomes (vitamin A pretreatment) inhibit the local hemorrhagic reaction normally produced in the skin of rabbits by combinations of bacterial endotoxin and epinephrine.

PUBLICATIONS

1. Janoff, A., Weissmann, G., Zweifach, B.W. and Thomas, L. Pathogenesis of Experimental Shock. IV. Studies on lysosomes in normal and tolerant animals subjected to lethal trauma and endotoxin. J. Exp. Med. 116:451-466, 1962.

2. Janoff, A. Alterations in lysosomes (intracellular enzymes) during shock. Effect of conditioning and protective drugs. Int. Anesthes. Clinic Symposium, New York, Little, Brown & Co. (in press).

II. VASOACTIVE POLYPEPTIDES - SVPx

During the past year we directed our efforts mainly on three aspects of the problem: (1) to substantiate the existence of SVPx as a distinct entity by differentiating it from other well-documented active substances; (2) to prepare and stockpile an amount of SVPx which would permit elucidation of its structure, and a systematic study of its pharmacological properties; (3) to initiate preliminary experiments towards elucidating the physiological role of SVPx and Helmer's protein.

1. SVPx as a discrete entity. Biological tests convincingly demonstrate that SVPx differs from noradrenaline, histamine, and serotonin. The active factor is dialyzable and is inactivated by proteolytic enzymes. A logical step then was

to compare SVPx with known biologically active polypeptides. These studies indicated that the vasoactive substance in plasma and serum is not to be accounted for by synthetic bradykinin, vasopressin, and oxytocin. SVPx differs also from synthetic angiotensin and eledoisin: although these two substances contract the aortic strip, they do so only partially and never to its maximal extent. Thus, despite the fact that angiotensin in nonagram amounts contracts the aortic strip, NOR and SVPx are more effective stimulants of vascular smooth muscle. Keele, et al., have described a pain-producing substance (PPS) in human plasma which has not been prepared in pure form. The serum vasoactive substance under investigation differs from PPS, in that it is not inactivated by human plasma. Helmer has reported on a plasma protein which acts as a potentiating principle and is found in dialyzed human plasma. This plasma factor strikingly increases the responses of the aortic strip to NOR. Some attention was given to the possibility that the plasma proteins might contain adsorbed SVPx, which could in effect act as Helmer's non-diffusible substance. This point has special significance inasmuch as Helmer subsequently found this non-diffusible substance to contract the aortic strip directly, particularly in blood from hypersensitive individuals. We were able to demonstrate without question that SVPx is not identical with Helmer's substance (5). (For full details on this work see attached manuscript.)

2. Efforts to prepare purified SVPx. SVPx is prepared in two stages. I. Dialysis of the rabbit serum or plasma against saline separates about half of the total SVPx content from the protein fraction. II. The protein-free dialyzate is purified by lyophilizing the dialyzate, and then eluted with boiling absolute alcohol. This step provides a NaCl-free active substance which is subsequently further purified by paper-chromatography. Location of the activity on the paper is achieved by (1) ninhydrin color reaction; (2) by cutting out selected paper

strips and testing them biologically; (3) by UV lamp fluorescence. The paper chromatography step is then repeated two additional times. Further efforts to prepare purified and concentrated SVPx are handicapped by losses of activity of the concentrated samples of SVPx, in spite of its remarkable stability to boiling, boiling in dilute acid, and standing.

3. Physiological role of SVPx and Helmer's protein. Only preliminary experiments have been made in this direction. It is clear from the above presentation that plasma, both of rabbits and humans, contains two vasoactive substances (SVPx and Helmer's substance) which have not been studied systematically before. We have found it possible to quantitate the amount of Helmer's substance in plasma on the basis of the in vitro potentiating action of the dialyzed plasma. Most probably both substances interfere with the biological assay of histamine and other substances in plasma and serum. In preliminary experiments we attempted (1) to determine normal values for both substances in human and rabbit plasma; (2) to assay for SVPx and Helmer's substance in hypertensive humans, and (3) to study the smooth muscle activity of lyophilized plasma fractions (Cohn procedure) in order to ascertain whether any of the isolated fractions could account for the overall effect of whole plasma. The results of these preliminary experiments will be reported upon completion.

PUBLICATIONS

1. Wurzel, M. On a vasoactive peptide (?) in the rabbit serum (SVPx). Fed. Proc. 21:112, 1962.

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3. Wurzel, M. and Zweifach, B.W. Differentiation of SVPx, a rabbit serum vasoactive peptide (?) from other biologically active peptides. Fed. Proc. 22:542, 1963.

4. Wurzel, M. and Zweifach, B.W. Differentiation of SVPx, a rabbit serum vasoactive peptide (?) from other biologically active peptides. (In preparation, copy enclosed).

5. Helmer, O.M. Differentiation between two forms of angiotensin by means of spirally cut strips of rabbit aorta. Am. J. Physiol. 188:571, 1957.

6. Wurzel, M., Pruss, T., Weiss, W., Maengwyn-Davies, G.D. Modification of rabbit aortic strip technic for catecholamine (4-point) assay and pharmacological studies. Proc. Soc. Exp. Biol. Med. 105:659, 1960.

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