Influence of Inositol on Peripheral Vascular Responses to Cold Injury. (22939)

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In the study of the effects of cold injury on living tissues a great deal of attention has been centered on vascular disturbances. Loss of plasma and packing of blood cells, as a result of cold injury, eventually lead to intravascular agglutinative thrombi and vascular failure(Greene, 1). It has been indicated that loss of plasma and consequent stasis is due to alteration of permeability of capillary walls(Fuhrman and Crismon, 2). Furthermore, vascular responses in areas remote from the injury are caused by toxic substance originating in the damaged tissues(Lewis, 3). Additional evidence that vascular constrictions and capillary damage are produced by a blood-borne substance has been presented by Sullivan and Kelley(4). Numerous attempts have been made to offset the effects of cold injury. Rapid rewarming of damaged tissues and anticoagulant therapy have been widely used but with controversial results. The present investigation involves the use of inositol, which combines anticoagulant activity with the property of protecting against capillary fragility. The antithromboplastic action of inositol was demonstrated by Kay and Balla(5). The fact that it decreases capillary fragility was reported by Belli, *et al.*(6). Both of these factors were taken into consideration in conducting this investigation. Observations were made to ascertain whether inositol can inhibit formation of agglutinative thrombi, reduce plasma loss by its capillary-protective action, and consequently, prevent stasis in peripheral vascular areas remote from cold injured tissues.

Materials and methods. Mature hamsters (*Mesocricetus auratus*) weighing 85 g to 120 g were used. Anaesthetization was achieved by intraperitoneal injection of Nembutal sodium (Abbot, 50 mg/cc). An initial dosage of 0.2 cc was administered, with additional dosages of 0.15 cc if required. Anaesthetized animals were placed on plexiglass microscope stage and the cheek pouch was everted and prepared according to the technic of Fulton, *et al.*(7). A small, incised flap of the pouch was spread over a glass block and pinned to paraffin moat exposing thin layer of tissue for microscopic examination. During preparation of the pouch and in the ensuing period of observation, the exposed tissues were continuously irrigated with physiological saline buffered to pH 7.4, and maintained at constant temperature of 37.5°C. The vascular components of the pouch were examined under magnifications of 100x to 200x. Observations were made on 4 series of animals; 1) normal, untreated controls, 2) cold injury controls, 3) those subjected only to inositol injections, and 4) those receiving inositol 10 minutes prior to induction of cold injury. All animals received similar doses of Nembutal and were under observation for 2.5 to 3 hours. Cold injury was produced by wrapping a hind limb to the knee joint in an aluminum foil boot containing pulverized CO_2 ice. It was held in place for one minute. Inositol was administered by intraperitoneal injection of 1 mg/100 g body weight. Inositol was dissolved in buffered saline and injected at 37.5°C.

Results. No spontaneous variation in diameter of the vessels was observed in untreated controls under the experimental conditions. Rate of flow was constant, although slightly more rapid on the arterial side of capillaries than on the venous side. A marked laminar flow was evident in arteries, but a more uniform flow was observed in veins. The direction of flow was subject to occasional fluctuations.

In cold injury controls the typical three-phase response(Sullivan, 6) was apparent. Application of dry ice to the hind limb produced (1) an immediate generalized increase in rate of blood flow. This was followed, after removal of the cold source, by (2) a

gradual decline in rate of flow. The final phase (3) observed 10-20 minutes after onset of thaw, was characterized by marked arteriolar constrictions, coatings of blood cells on the venous endothelium, formation of thrombi, retardation of venous flow, intravascular packing of blood cells, and stasis.

In animals subjected only to inositol injections there were no deviations from the normal vascular pattern and reactions. However, in the preparation of the cheek pouch these animals revealed a marked tendency to profuse hemorrhage when even a small vessel was damaged.

In animals treated with inositol before induction of cold injury the initial phase of rapid flow and the secondary gradual decline were apparent. There was no deviation from cold injury controls in either degree of change or time factor. The constriction phase appeared in the usual time after onset of thaw (10-20 minutes). Sluggish flow was apparent in veins but this condition was not as prominent as in cold injury controls. A slight endothelial coating of cellular elements was observed in some of the smaller venules but this was not as widespread nor as severe as in the controls. The number of functional capillaries was somewhat reduced but in the open vascular beds the rate of blood flow appeared normal. In general, there was a definite alleviation of the conditions which normally follow cold injury, and no case of complete stasis was observed in the inositol treated animals.

Discussion. It has been pointed out that peripheral vascular responses to cold injury are due primarily to loss of plasma through impaired capillary walls(2) and the formation of agglutinative thrombi(8). These conditions lead to intravascular packing of blood cells, occlusion of vessels, and ultimately, complete stasis. Local cold injury suffered by the tissues is dependent specifically upon the extent of these conditions and the resultant tissue anoxia(Shumacker,9). From a theoretical viewpoint it would seem that any agent which prevents or ameliorates these vascular disturbances would promote and maintain a more adequate blood flow, reduce anoxia, and decrease tissue damage.

The results of the present investigation indicate that inositol has the requisite properties of decreasing capillary impairment, inhibiting formation of thrombi, and preventing post-constriction stasis. Consequently, an adequate blood volume is maintained and the usual post-thaw packing of vessels with stranded cells does not occur. Presumably, the improved peripheral blood flow increases the supply of oxygen to the injured tissues, and despite the increased metabolic rate, should result in less necrosis.

Summary. Control animals receiving inositol injections showed no deviation from the normal vascular pattern or reactions. However, the concentration of inositol administered (1 mg/100 g of body weight) markedly increased the tendency to hemorrhage. When inositol was injected into animals prior to induction of cold injury the initial responses of frostbite controls occurred without deviation. However, the constriction phase was ameliorated, thrombi formation was inhibited, and complete stasis was eliminated.

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