Differences in Concentrations of Acid Mucopolysaccharides Between Spleens of Normal and Polycythemic CF_1 Mice[1] (37641)

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(Introduced by Roger C. Crafts)

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Recent investigations have suggested that the microenvironment of a hemopoietic organ may influence the cellular development within that organ. For example, Trentin (1) has proposed a concept of hemopoietic inductive microenvironments (HIM) which determine the line of differentiation to be followed by the pluripotential stem cells. Patt and Maloney (2) have suggested that local regulatory mechanisms are operative in cellular proliferation in the bone marrow, and the importance of the stroma in erythropoiesis also has been emphasized by Brecher *et al.* (3).

Recently, McCuskey *et al.* (4) have reported changes in the murine splenic microenvironment during erythropoietic suppression and regeneration and considered the microenvironment to be divided into three compartments: a microvascular compartment composed of arterioles, capillaries, sinusoids, and venules; a connective tissue compartment composed of cells, fibers, and ground substances; and the neural elements associated with the blood vessels and the stroma. In these studies, differences were found *in vivo* between the microcirculation of erythropoietically-active spleens of normal mice and the erythropoietically-suppressed spleens of polycythemic mice. The spleens of polycythemic mice had limited blood flow through the splenic sinusoids with most sinusoids storing blood. Histochemical analysis of the connective tissue compartment indicated that the red pulp of the erythropoietically-suppressed spleens contained predominantly acid mucopolysaccharides while that of normal spleens contained predominantly neutral mucopolysaccharides. From these results, McCuskey *et al.* (4) hypothesized that erythroid development could be completed only in a microenvironment which had adequate blood flow and which contained neutral mucopolysaccharides. In contrast, a microenvironment which contained high concentrations of acid mucopolysaccharides and limited blood flow was not conducive for erythropoiesis. It was further hypothesized that the decreased blood flow, observed in the erythropoietically-suppressed spleens, might produce local hypoxia which, in turn, was responsible for the change in the type or concentration of mucopolysaccharides in the connective tissue compartment of the microenvironment.

Since the reported changes in mucopolysaccharides were based on qualitative histochemical methods, it was thought necessary to confirm and quantify these changes biochemically. Accordingly, two specific acid mucopolysaccharides, hyaluronic acid and chondroitin sulfate, were isolated from spleens of normal and polycythemic mice and the concentrations of these two substances were quantified.

Materials and Methods. Female CF_1 mice weighing 19–20 g were used as the experimental animals. The animals were divided into two groups: a nontransfused group, with active erythropoiesis, and a polycythemic group with suppressed erythropoiesis. Erythropoiesis was suppressed by transfusion on two consecutive days with ip injections of 0.8

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ml of packed, saline-washed red blood cells and the animals sacrificed 6 days later. The spleens were selected from control animals having hematocrits between 38–52%; and from polycythemic mice with hematocrits greater than 60%. Only spleens which weighed between 80–200 mg were used. Spleens were excised, weighed, immediately frozen, and dehydrated. For assay purposes, the spleens were further divided into six groups containing 15–20 spleens each; 4 for the control and 2 for the polycythemic animal.

After tissues were defatted and dried, acid mucopolysaccharides were extracted by an adaptation of the method of Schiller *et al.* (5). Acid mucopolysaccharides were separated as cetylpyridinium chloride (CPC) complexes into hyaluronic acid and chondroitin sulfate fractions by a modification of the method of Schiller *et al.* (5). The CPC was removed by precipitation with 3 *M* NaCl and the fractions were dialyzed to remove the last trace of salt. The relative content of acid mucopolysaccharides in the fractions were estimated by determination of uronic acid by the carbazole method of Dische (6) using D-glucuronic acid as the standard.

Results. The concentrations of acid mucopolysaccharides in the spleens of control animals with active erythropoiesis was found to differ from that seen in the spleens from polycythemic animals in which erythropoiesis was suppressed (Table I). The mean concentrations of hyaluronic acid in the polycythemic group was 55% greater than that in the control group (130.5 μg and 84.5 μg respectively). The mean concentration of the chondroitin sulfate in the polycythemic groups was 26% greater than that of the control groups (131.0 μg and 104.5 μg, respectively). These differences were found to be statistically significant according to the Fisher *t* test for significance.

Discussion. Mucopolysaccharides are recognized as a component of the extracellular ground substance in connective tissue. Because all substances going to and from cells must pass through this extracellular compartment, changes in its state and composition may exert a significant influence on individual cells and on the tissue as a whole. For example, it has been suggested that mucopolysaccharides play a significant role in the initiation and control of cellular division (7), in cellular aggregation (8), and in hair growth (9). Dorfman (10) suggested that mucopolysaccharides of connective tissues have a role in a number of physiological and pathological processes including calcification, control of electrolytes and water in extracellular fluids, wound healing, lubrication, and the maintenance of the stable transport medium of the eye.

TABLE I. Concentrations of Hyaluronic Acid (HA) and Chondroitin Sulfate ($ChSO_4$) in Spleens of Normal and Polycythemic Mice.[a]

Fraction	Control[b]	Polycythemic[b]	p[c]
HA	76.0	117.0	0.01
	89.0	144.0	
	94.0	(130.5)	
	79.0		
	(84.5)		
$ChSO_4$	108.5	139.0	0.02
	110.0	123.0	
	105.0	(131.0)	
	94.5		
	(104.5)		

[a] Expressed as μg uronic acid per g lipid-free dry weight.

[b] Each value represents analysis of 15–20 pooled spleens. The mean is given in parentheses.

[c] Probability was calculated from a *t* test between the control and polycythemic groups.

That the mucopolysaccharides might play a role in the process of erythropoiesis has been suggested by the studies of McCuskey *et al.* (4, 11). The present biochemical demonstration of changes in the concentrations of two acid mucopolysaccharides between spleens of normal and polycythemic CF_1 mice substantiates and quantifies their histochemical findings. Whether or not these changes are related to tissue oxygenation, as suggested by McCuskey *et al.* (4, 11), remains to be determined. However, similar changes have been reported in the aortic wall following hypoxia (12).

Summary. Biochemical analysis of the spleen demonstrated that when erythropoiesis is suppressed there is a significant increase in the concentration of two acid mucopolysaccharides, hyaluronic acid and chondroitin

sulfate. These results support previous histochemical studies which suggested that mucopolysaccharides may play an important role in erythropoiesis.

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