Study of Different "Fibrinoids" by Histochemical Means.* (22466)

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The so-called fibrinoid degeneration occurs in a variety of pathological conditions. It is characterized by the appearance of a homogeneous, strongly acidophilic material which is stained by Weigert's fibrin and the P.A.S. methods. The problem whether fibrinoid change is due to impregnation of the tissue by fibrin, or represents a change in the connective tissue ground substance has not yet been settled definitely(1-4); neither is it clearly known whether the "fibrinoid materials" differ in the different pathological states in which they occur (with some common characteristics which are responsible for the common staining reactions), or whether it is a single compound appearing in a variety of diseases(5,6).

The present report deals with attempts to answer these two questions by a study of the histochemical characteristics of fibrinoid materials present in different diseases.

Materials and methods. Formalin fixed and paraffin embedded human autopsy and biopsy material was used. The choice of material was casual and bore no relationship to etiological and pathogenetic factors. Normal fibrin was included for comparison with the "fibrinoids." The following specimens were included in all experiments. (1) Mural thrombus of heart; (2) three month placenta; (3) placenta at term; (4) nephrosclerosis with signs of malignant hypertension; (5) heart in systemic lupus erythematosus; (6) kidney in periarteritis nodosa; (7) pinguecola in the conjunctiva. Other examples of fibrinoid change were included originally in the series, but owing to the small size of blocks they had to be subsequently discontinued. Serial sections 8 μ in thickness, were made from all the blocks. The different staining and histochemical blocking procedures were performed simultaneously on slides of each tissue. The following staining methods were used: a) H. & E.; b) Weigert's fibrin method; c) Mallory's Azan; d) periodic acid Schiff method (P.A.S.); e) Rinehart and Abul Haj's modification of Hale's colloidal iron method; f) ninhydrin-Schiff method; g) performic-acid-Schiff; h) Sudan black; i) Weil-Weigert's myelin method; j) mucicarmin. The effect of the following histochemical blocking reactions and treatments on staining by the 4 first mentioned staining procedures was studied; a) acetylation for 24 hours at 37° with an acetic anhydride-pyridine mixture; b) deacetylation of the acetylated sections; c) deamination by van Slyke's nitrous acid reagent; d) deamination by ninhydrin; e) extraction of sections for 24 hours by 1% acetic acid; f) extraction for 24 hours by pyridine; g) extraction for 24 hours by 1% sodium carbonate. All the histological and histochemical procedures were performed as suggested by Lillie(7).

Results. Table I summarizes the differences in staining of the various "fibrinoids" by the ninhydrin-Schiff and the colloidal-iron staining reactions.

The ninhydrin-Schiff staining reaction indicates the relative concentration of protein matter (as represented by α amino acids) within the "fibrinoids," while the colloidal iron reaction visualizes acidic groups which probably belong to mucopolysaccharides. With the fibrin in the mural thrombus serving as control, it can be deduced that the eosinophilic material surrounding the villi and the Nitabuch layer in both the young and mature placentas contains protein in a similar concentration to that of fibrin. The "fibrinoid" of renal arteriosclerosis and of periarteritis nodosa appears to contain more protein material, while that of pinguecola contains much less protein than the fibrin. With the colloidal iron technic more marked differences can be noted between the fibrin and all the

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TABLE I. Staining of Different "Fibrinoids" by the Ninhydrin-Schiff and the Colloidal Iron Technics.

			Lupus erythematosus			Mature placenta		Incomplete abortion		
	Pinguecola	Periarteritis nodosa	"Fib'n" on pericardium	"Fibrinoid" in artery	Renal arteriolosclerosis	"Fibrin" around villi	Nitabuch's layer	"Fibrin" around villi	Nitabuch's layer	Fibrin in mural thrombus, heart
Ninhydrin-Schiff	±	4+	?	?	4+	2+	2+	2+	2+	2+
Colloidal iron staining	4+	2+	?	?	2+	3+	3+	3+	3+	+

Note: Intensity of the staining of "fibrinoids" was graded as: 0 = no staining; ± = staining barely visible; + = weak; 2+ = moderate; 3+ = marked; 4+ = intense staining.

"fibrinoids." The pinguecola "fibrinoid" appears to contain most acidic groups, while that of renal arteriosclerosis and of periarteritis nodosa does not differ much from fibrin. The difference in staining by colloidal iron between the Nitabuch layer and the "fibrin" which surrounds the placental villi on the one hand, and the fibrin of the thrombus on the other, is remarkable.

It has been stated that acidophilia is due to the presence of free amino groups(8). Accordingly, a study was made of the effect of reagents which block or destroy the amino groups, on the staining of different "fibrinoids" with the H.&E. and the Azan staining methods. It can be seen in Table II that the effects of the blocking agents were comparable, although not identical, for both staining methods. Treatment by van Slyke's reagent was the most effective in blocking acidophilia. It inhibited completely the staining of fibrin and of the "fibrin" around the villi, the "fibrin" of the pericarditis in Lupus erythematosus, and of the "fibrinoid" of pinguecola. The treatment did not completely block, however, the acidophilia of the other "fibrinoids." Treatment by ninhydrin was less effective in blocking acidophilia, but the materials which behaved like fibrin were the same for both blocking methods. Acetylation was less effective than van Slyke's reagent, but more effective than ninhydrin in abolishing acidophilia. The four compounds, which behaved like fibrin towards the other 2 amine-blocking procedures after acetylation, stained with the same intensity as fibrin.

Two materials were affected differently from the others by the amine blocking procedures. (1) The "fibrinoid" of pinguecola, which behaved like fibrin towards the van Slyke reagent, was not affected by the acetylation. (2) The acidophilia of the "fibrinoid" of periarteritis nodosa was affected to the same extent by ninhydrin treatment as by

TABLE II. Effect of Varied Treatments as Indicated by Subsequent Staining of Different "Fibrinoids" by Acid Dyes (H. & E. and Azan's Methods).

			Lupus erythematosus			Mature placenta		Incomplete abortion		
Effect of	Pinguecola	Periarteritis nodosa	"Fib'n" on pericardium	"Fibrinoid" in artery	Renal arteriolosclerosis	"Fibrin" around villi	Nitabuch's layer	"Fibrin" around villi	Nitabuch's layer	Fibrin in mural thrombus, heart
A*	4+	2+	2+	4+	3+	2+	3+	2+	3+	2+
A and D*	4+	4+	2+	4+	3+	2+	3+	2+	3+	2+
Van Slyke's R*	0	2+	0	2+	+	0	2+	0	2+	0
Ninhydrin R*	3+†	2+†	3+†	3+†	4+†	3+†	4+†	3+†	4+†	3+†

Note: Intensity of the staining of "fibrinoids" was graded as: 0 = no staining; ± = staining barely visible; + = weak; 2+ = moderate; 3+ = marked; 4+ = intense staining.

* A = Acetylation; D = Deacetylation; R = Reagent.

† Findings with Azan; effect on eosin staining was much more pronounced.

TABLE III. Effect of Varied Treatments as Indicated by Subsequent Staining of Different "Fibrinoids" by Weigert's Fibrin Method and by the P.A.S. Procedure.

			Lupus erythematosus			Mature placenta		Incomplete abortion		
Effect of	Pinguecola	Periarteritis nodosa	"Fib'n" on pericardium	"Fibrinoid" in artery	Renal arteriolosclerosis	"Fibrin" around villi	Nitabuch's layer	"Fibrin" around villi	Nitabuch's layer	Fibrin in mural thrombus, heart
A*	0	0	0	0	0	0	0	0	0	0
A and D*	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
Van Slyke's R* on fibrin stain	4+	4+	4+	4+	3+	3+	3+	4+	4+	3+
Van Slyke's R* on P.A.S.	+	2+	4+	4+	4+	3+	3+	3+	3+	2+
N* on fibrin stain	3+	2+	?	?	2+	0	2+	0	+	0
N* on P.A.S.	+	2+	+	3+	4+	2+	4+	+	3+	+

Note: Intensity of the staining of "fibrinoids" was graded as: 0 = no staining; ± = staining barely visible; + = weak; 2+ = moderate; 3+ = marked; 4+ = intense staining.
* A = Acetylation; D = Deacetylation; R = Reagent; N = Ninhydrin.

van Slyke's reagent. It was also affected by acetylation to the same extent as fibrin.

The "deacetylation" of acetylated sections had no effect on acidophilia in most materials. The only marked effect in restoring acidophilia was noted in the "fibrinoid" of periarteritis nodosa. It should be noted, though that no such effect could be studied where the acetylation itself had no effect on acidophilia.

Table III shows the results of different procedures on the P.A.S. and Weigert's fibrin staining methods. It is noteworthy that Weigert's method reacted similarly to P.A.S. towards the blocking agents. As both methods were completely blocked by the acetylation procedure and could be reversed by deacetylation, it is believed that staining by Weigert's fibrin method might be due to the presence of 1.2 glycols or 1-glycol-2-amino groups. The implications of these findings in the problem of Gram staining of bacteria are obvious as the Gram procedure is almost identical with Weigert's fibrin method.

Deamination by van Slyke's reagent did not markedly affect the staining of the "fibrinoids" by Weigert's fibrin stain, but it did influence to different degrees the staining of the various "fibrinoids" by P.A.S. The "fibrinoid" of periarteritis nodosa behaved in this case like fibrin. The "fibrinoid" of pinguecola was even more affected by van Slyke's reagent than fibrin. Van Slyke's reagent had no or little effect in this staining with any of the other "fibrinoids."

By the effect of ninhydrin treatment on both Weigert's fibrin and P.A.S. methods the "fibrinoids" could be divided into 3 groups. The "fibrin" around the placental villi and the fibrin in the pericardium in Lupus erythematosus behaved like fibrin. All the other "fibrinoids" with the exception of that of pinguecola, behaved differently. Again, the "fibrinoid" of pinguecola was unique in its behavior.

No relevant additional findings were obtained by the use of the other staining and blocking methods.

Discussion. The data reported above indicate that the term "fibrinoid" is merely descriptive and covers a number of different compounds. The various "fibrinoids" differed from fibrin to a varying extent. The "fibrin" layers around the placental villi and in the pericardium of Lupus erythematosus were most similar to the fibrin of the mural thrombus. It is possible that we are dealing in this case with fibrin in which a change has occurred. The hypothesis which derives "fibrinoid" from plasma fibrin(3) does not seem to hold for the other "fibrinoids" studied in this work. The common staining characteristics of the different fibrinoids appear to

be due to the presence of saccharidic complexes. The common denominator of all these compounds is their acidophilia and their staining by Weigert's fibrin and P.A.S. methods. These reactions might be due to free amino-groups, many of which are probably in a 2 position in respect to hydroxyls (as the deamination procedures had some effect on the P.A.S. staining). It seems probable, although it has not been proved, that the complexes responsible for the staining reactions of the fibrinoids and fibrin might be different polysaccharides, possibly poly-amino-sugars (1). Our findings corroborate those of other workers who found that "fibrinoids" differed from fibrin in their histochemical(1,6,8,9), physical(10), and biochemical(11) characteristics.

The multiplicity of "fibrinoids" confirms and extends the views expressed by Klemperer(5) and by Gueft and Laufer(12) who maintained that the systemic Lupus erythematosus fibrinoid derives from DNA and is therefore chemically different from the other "fibrinoids."

Summary and conclusions. 1. The types of "fibrinoids" in different pathological processes were studied by means of specific staining reactions and histochemical procedures intended to block amino groups. 2. All fibrinoids differed from fibrin in their behavior. The "fibrin" layer around the placental villi behaved more like fibrin than any other "fibrinoids." The other "fibrinoids" differed markedly from each other and from fibrin. It may be concluded that in spite of their tinctorial similarities, the various "fibrinoids" contain different compounds and might represent the end-stages of different processes.

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Effect of High Hydrostatic Pressures on Colchicine-Damaged Mast Cells of Peritoneal Fluid.* (22467)

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Morphologic changes in the mast cells of rat peritoneal fluid have been induced by subcutaneous injections of colchicine(1). The cells, normally spherical, are altered into irregular masses, sometimes with branched pseudopod-like extensions of cytoplasm, and the nucleus appears to be pushed to the periphery of the granular cytoplasmic mass as if being ejected from the cell. These changes

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