IMMUNOLOGIC STUDIES OF HEART TISSUE

III. Occurrence of Bound Gamma Globulin in Auricular Appendages from Rheumatic Hearts. Relationship to Certain Histopathologic Features of Rheumatic Heart Disease*, ‡

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Plates 1 to 4

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The present work gives evidence by use of fluorescent antibody methods of deposits of *bound* gamma globulin with characteristic histologic distribution in the auricular appendages of certain patients with rheumatic heart disease. These deposits were not associated with Aschoff lesions; they occurred in spotty distribution throughout the myocardium, involving myofibers, and to a lesser extent, connective tissue and vessel walls. Other proteins, *i.e.*, albumin and fibrinogen, were absent from these foci. Furthermore, the tissue sites demonstrating fixation of gamma globulin frequently exhibited evidences of alteration as indicated by affinity for eosin and intense reactivity with the periodic acid–Schiff stain. *Bound* gamma globulin with associated histochemical change was observed in a significant proportion of rheumatic hearts, but not in normal or pathologic control tissues.

In previous studies of rheumatic hearts, Vazquez and Dixon (1) described in fixed tissue sections gamma globulin in altered perivascular connective tissue and in Aschoff lesions. According to the authors, "it was not possible to show a difference between the amount of gamma globulin visualized specifically within the fibrinoid necrosis of the Aschoff bodies and in the immediate

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surrounding edematous connective tissue." Less staining was noted for albumin than for gamma globulin. Involvement of myofibers and vessel walls was not described. In other studies of lesions of connective tissue disease, deposits of gamma globulin have been found associated with fibrinoid alteration of arteries in disseminated lupus and in periarteritis (2, 1), in the zone of central necrosis in rheumatoid subcutaneous nodules (1), and in the wire loop lesion of the lupus kidney (3). These various reports have been reviewed recently (4).

The data to be presented are based on a survey of 100 biopsied auricular appendages from patients with rheumatic heart disease. The histologic distribution of bound gamma globulin, its association with histochemical change, and the relationship of these findings to certain histopathologic features of rheumatic heart disease are described.

Materials and Methods

The rheumatic tissue specimens surveyed in this work included 100 biopsied auricular appendages[1] from patients judged clinically inactive. Control tissue from pathologic as well as normal hearts was obtained from 30 hearts at postmortem and from 3 auricular biopsies. The pathologic control tissues exhibited myocardial fibrosis, mild to severe, in 9 cases; acute myocardial infarction in 4, pericarditis in 2, interstitial fibrosis in 1, and myxoma of left auricle in 1. The remaining control tissues were considered normal. Two of the auricular biopsies were from hearts with congenital defects; the third was from the case of myxoma.

Pieces of each specimen, 1 cm. on edge, were quick-frozen in alcohol-dry ice mixture, and stored at −30°C. before sectioning in a cryostat. Companion pieces were fixed in formalin. Sections from unfixed frozen and formalin-fixed paraffin-embedded tissues were stained with hematoxylin and eosin, phosphotungstic acid-hematoxylin, and with periodic acid–Schiff reagent by the McManus technique (5).

Immunofluorescent Method.—Antihuman gamma globulin was prepared in rabbits using as antigen, a highly purified human gamma globulin preparation (Lot II-G-OLI) estimated to contain less than 1 per cent contaminating protein by electrophoresis.[2] The antigen solution was absorbed on aluminum hydroxide gel and immunization carried out by repeated subcutaneous injection. The resulting antiserum tested against normal human serum by double diffusion in agar yielded a single line giving a pattern of identity with the precipitin line against normal 7S gamma globulin. Antiserum to purified human albumin[2] was similarly prepared. The globulin fractions from these antisera were labeled with fluorescein isocyanate as previously described (6), or with fluorescein isothiocyanate (7) in the absence of organic solvents.

The antifibrin conjugate was the same preparation employed by Gitlin[3] and associates in their studies of fibrinogen and/or fibrin distribution (8). The term fibrin is used in this paper, as by these authors, to refer to either or both proteins.

Removal of "non-specific" staining properties from these conjugates was accomplished by

[1] These tissue specimens were kindly made available by Dr. Dwight E. Harkin and Dr. Gustave Dammin, Peter Bent Brigham Hospital, Dr. Gordon Scannell and Dr. Lawrence Kunz, Massachusetts General Hospital, Boston, and Dr. George H. A. Clowes, Metropolitan General Hospital, Cleveland.

[2] We are grateful to Dr. L. J. Oncley and Dr. D. M. Surgenor, Department of Physical Chemistry, Harvard Medical School, for generous gifts of preparations of gamma globulin, albumin, and fibrinogen.

[3] We are indebted to Dr. D. Gitlin for a generous supply of this preparation.

absorption with mouse liver powder (6). The antifibrin conjugate had been absorbed with rat liver powder and rabbit bone marrow powder.

Tissue sections were cut at 4 mμ in a cryostat, dried for at least 1 hour in a draft of a fan before using, and were washed free of the soluble proteins distributed in the tissues without prior fixation in organic solvents.

The details of the staining procedure are as follows: The air-dried slides were immersed in buffered saline (0.01 M phosphate, pH 7.2) and washed for 20 minutes in two changes of this buffer with continuous agitation. After wiping the slides free of excess moisture, 1 to 2 drops of conjugate were applied, and the sections stained for 1 hour in a moist chamber. The slides were then washed for 10 minutes in two changes of buffered saline and mounted in glycerol-buffer. Under these conditions, neither gamma globulin nor albumin could be detected in normal heart tissue sections; while fibrin, presumably converted from fibrinogen, could be detected in variable amounts. Gamma globulin or albumin observed in tissues after this washing procedure were designated as "bound," for the purposes of this work.

The ultraviolet source was a Zeiss lamp fitted with an Osram HBO-200 mercury lamp, filtered with 2 half-thicknesses of Corning filter 5840, and a red-excluding filter BG-14.

Proof of immunologic specificity of staining was based on the following control observations: (*a*) Failure of reaction after specific absorption of the conjugate with purified antigen in slight excess; (*b*) positive inhibition tests with unlabeled antisera (6); (*c*) failure of reaction with heterologous conjugate or with fluorescein-labeled normal rabbit serum.

Certain of the sections showed marked non-specific reactivity with conjugate at sites of eosinophilic alteration. Staining of these sections with conjugate adjusted to pH 8.0, using buffer washes at pH 8.0, was found to suppress almost completely this nonspecific reactivity without significant reduction in intensity of the specific immunochemical reaction.

Histopathologic Study. Diagnosis of Aschoff Body.—The formalin-fixed paraffin sections were studied independently by one of the authors (F.D.D.) without knowledge of the immunofluorescent results. Criteria for the histopathologic diagnosis of the Aschoff body, often controversial (*cf.* 9–12) consisted of the following criteria: The description, "*active* Aschoff nodule," was used in reference to a round or ovoid granulomatous collection of cells associated with alterations of the connective tissue, which included basophilic staining of the intercellular matrix typical of mucoid degeneration, fragmentation, and swelling of collagen fibers giving brightly acidophilic staining, and fusion of such fibers with a central mass of fibrinoid necrotic substance. The inflammatory cells were usually numerous and consisted of mono- or multinucleated cells of Aschoff, large mesenchymal cells, lymphocytes, and other mononuclear elements. The diagnosis of *inactive* Aschoff lesion involved a broader range of histologic changes, and, in general, included lesions which were small and characterized by poorly delineated foci of dense hyalinized connective tissue and sparse infiltrates of chronic inflammatory cells. Giant cells were rare, and, when present, contained a dark pyknotic nuclear mass without fibrocytoid or stellate configurations. These latter lesions are analogous to Ticknap's "possible" Aschoff lesions (10), and to the "inactive" lesions of Tedeschi and Wagner (11).

RESULTS

Occurrence of Bound Gamma Globulin in Auricular Appendages of Rheumatic Hearts.—As shown in Table I, 18 of 100 auricular appendages exhibited significant deposits of bound gamma globulin. Definite but scanty amounts were observed in 12 other specimens; the remainder were negative. Control tissues, including pathologic and normal specimens, were negative in 32 of 33 cases.

The remaining specimen was an auricular biopsy from a heart with myxoma, which showed only traces of gamma globulin in interstitial connective tissue.

Histologic Distribution of Gamma Globulin.—Bound gamma globulin was observed in rheumatic hearts in a pattern of histologic distribution which involved, in order of relative frequency, myofibers, interstitial connective tissue, and vessels.

This histologic distribution may be observed in varying aspects in Figs. 1 to 12. Deposits were observed most frequently in segments of myofibers, usually involving sarcolemma and peripheral sarcoplasm. The focal concentrations of gamma globulin in these sites gave an appearance of fused droplets or amorphous masses spreading from the margins of the myofiber into the substance of the sarcoplasm, as illustrated in Figs. 1 to 4 and 6 to 9. While sub-

TABLE I

Occurrence of Bound Gamma Globulin in the Myocardium of Rheumatic Hearts

Tissue source	No. of tissue specimens	BGG* present	BGG present in traces	BGG absent
Rheumatic hearts				
Auricular biopsies	100	18	12	70
Non-rheumatic hearts‡	33	0	1	32

* BGG, bound gamma globulin, defined as that demonstrable in unfixed washed tissue sections.

‡ Of these 33 non-rheumatic hearts, 30 were postmortem specimens, and 3, auricular biopsies.

sarcolemmal distribution in the myofiber was seen most frequently, rod-like condensations or diffuse amorphous masses were also seen in intermyofibrillar spaces (Figs. 1 to 3, 10 to 12). These involved myofibers were usually scattered in distribution, as illustrated in Figs. 1, 3, and 9. Rarely, penetration of a small sector of myocardium *en bloc* was noted, as in Fig. 10, with scattered distribution in adjacent regions (Fig. 17).

The stroma of the myocardium, endocardium, and epicardium, also exhibited focal concentrations of bound gamma globulin. These deposits could frequently be made out within the bundles of collagenous septae, or within the substance of collagen in areas of increased fibrous connective tissue (Figs. 1, 2, 7, 13, 17). Frequently, the deposits in the interstitium of the myocardium extended into, and became confluent with gamma globulin in sarcolemma (Figs. 1, 7). All of the 18 specimens positive for bound gamma globulin exhibited this material in myofibers and interstitial connective tissue. Gamma globulin was also observed in walls of small arteries, arterioles, and venules in 12 of these 18 specimens. These deposits were observed mainly in segmental portions of media

TABLE II

Comparative Incidence of Bound Gamma Globulin, Albumin, and Fibrin in Rheumatic Hearts. Correlation with Affinity for Eosin as Fluorochrome

	No.	Patient	Bound or insoluble protein present in washed section			Staining reaction with eosin as fluorochrome
			Gamma globulin	Albumin	Fibrin	
Rheumatic hearts	1	Gas	++++	0	Traces	++++
	2	San	++++	0	+	+++
	3	Cap	+++	0	+	++
	4	For	+++	−	+	++++
	5	Lau	+++	0	+++	++++
	6	Spi	+++	Traces	Traces	+++
	7	Woo	+++	+	+++	++++
	8	Hab	++	Traces	+	++
	9	Shu	++	0	0	++
	10	Sil	++	Traces	0	−
	11	DeA	+	0	+	++++
	12	Zam	+	0	+	++
	13	Bak	Traces	+	+	+
	14	Urb	Traces	Traces	Traces	++
	15	Den	Traces	Traces	+	+
	16	Fra	Traces	0	+++	+
	17	Mac	Traces	0	+	+
	18	Moo	Traces	Traces	+	0
	19	Tuo	Traces	+	+	Traces
	20	Rou	0	0	+	+++
	21	Bar	0	0	+++	0
	22	Cli	0	0	Traces	0
	23	Eil	0	0	0	−
	24	Fly	0	0	+	0
	25	Ise	0	0	+++	−
	26	Mag	0	0	+	0
	27	Ril	0	0	+	Traces
	28	Ros	0	0	++	−
	29	Sha	0	Traces	Traces	0
	30	Tib	0	0	+	0
	31	Vog	0	0	Traces	0
	32	Ulm	0	0	0	0
Non-rheumatic hearts	1	Bel	0	0	+	0
	2	Fl	0	0	+	0
	3	Gr	0	0	0	0
	4	Joh	0	0	Traces	0
	5	MacD	0	0	+	0
	6	Mey	0	Traces	++	0
	7	Pay	0	0	+	0
	8	Pol	0	0	Traces	0
	9	Win	0	0	+	0
	10	Ross	0	−	−	0

and intima, as illustrated in Figs. 5, 6, 14 to 16, 19. The extent of such vascular deposits varied considerably from specimen to specimen, and was never observed separate from involvement of myofibers and connective tissue. In a rare instance, as in Fig. 16, gamma globulin was seen in a segment of myofiber adjacent to an involved vessel; in general, however, distribution of bound gamma globulin in muscle and connective tissue showed no relationship to vessels.

Comparative Distribution of Gamma Globulin, Albumin, and Fibrin.—Sections from a series of 20 rheumatic hearts which contained varying amounts of bound gamma globulin were examined for presence of albumin and fibrin. As shown in Table II, albumin was absent from most of the specimens examined. In the few specimens where present, it occurred in minor or trace amounts in the interstitial connective tissue, unrelated to the distribution of bound gamma globulin.

In the case of fibrin, varying amounts of this material were observed in both rheumatic and non-rheumatic specimens (Table II). Several specimens revealed little or no material. Histologic distribution within both groups was similar, and involved mainly endothelium and adventitia of vessels, and scattered foci in interstitial connective tissue. As determined by comparison of adjacent sections, the histologic distribution of fibrin and bound gamma globulin was unrelated except for occasional overlapping in vessels or in interstitial connective tissue (Figs. 17 and 18).

Evidence of Histochemical Change at Sites of Gamma Globulin Localization.—In certain of the rheumatic hearts with bound gamma globulin, sections stained with hematoxylin-eosin gave evidence of foci with intense eosinophilic homogeneous refractile staining. These eosinophilic sites usually occurred in segments of sarcolemma and myofiber sarcoplasm. Occasionally entire myofibers or myofiber bundles were involved. Small arteries, arterioles, and venules similarly exhibited refractile, intensely eosinophilic foci in their walls, with fusion or clumping of smooth muscle elements. This affinity for the eosin stain could be demonstrated with remarkable definition by using dilute eosin (tetrabromofluorescein) as a fluorochrome stain, at a concentration found unreactive with normal heart tissue.

This eosin fluorochrome stain was carried out as follows:

> Unfixed or acetone-fixed and dried sections were immersed for 20 minutes in a dilute solution of eosin prepared by adding 0.1 ml. of a 0.5 per cent alcoholic solution of eosin to 100 ml. of buffered saline, pH 7.2. The sections were then washed in two changes of buffered saline for 20 minutes, with continuous shaking, and mounted in glycerol buffer. Structures exhibiting affinity for eosin emitted a brilliant yellow-orange fluorescence, which tended to quench on prolonged exposure to ultraviolet light.

The histologic sites stained by eosin in most of the rheumatic hearts corresponded fairly closely with the pattern of distribution of bound gamma globulin

in myofibers, sarcolemma, interstitial connective tissue, and vessels, as illustrated in Figs. 20 to 24. This correlation may be observed also in Table II. The fluorochrome reaction usually gave a more widespread pattern, with staining of finer histologic structures than noted with the anti-gamma globulin conjugate. In three instances, however, gamma globulin was sparse or absent in specimens exhibiting extensive fluorochrome staining (specimens DeA, Urb, and Rou) as shown in Table II. Thus, while gamma globulin deposition and eosinophilic alteration were usually found associated, these observations indicated that eosinophilic change might infrequently be observed in the absence of gamma globulin. It was of interest that fibrin was not stained by the eosin fluorochrome either in rheumatic or non-rheumatic specimens.

Additional evidence of tissue alteration at sites of localization of bound gamma globulin in sarcolemma, myofiber sarcoplasm, interstitial connective tissue, and vessel walls was the intense reaction given by these same sites when the sections were stained with the periodic acid–Schiff reagent. Those specimens with little or no detectable gamma globulin which showed intense eosinophilic alteration also exhibited this enhanced reaction with the PAS reagent at the eosinophilic sites.

Histopathologic Observations and Correlation with Immunofluorescent Findings.—Both unfixed frozen and routine paraffin sections from each specimen were surveyed for the following changes: focal myocardial cell necrosis, waxy degeneration of myofibers, myocardial fibrosis, presence of Aschoff bodies, fibrinoid and other alterations of collagen, involvement of vessels, and interstitial inflammation.

As noted previously, myofibers containing bound gamma globulin frequently showed refractile eosinophilic clumps or masses within the sarcoplasm. These changes were observed most often in the absence of inflammatory response. Occasionally, infiltration by polymorphonuclear and mononuclear cells was observed. Involved vessels showed intensely eosinophilic segments of walls in which smooth muscle and ground substance appeared fused in a homogeneous refractile mass, consistent in appearance with fibrinoid alteration. Little or no inflammatory response was associated with these changes. The scattered deposits of gamma globulin in connective tissue of endocardium, epicardium, and in collagenous septae were also associated with eosinophilic alteration of these sites generally without inflammatory or cellular response. Bound gamma globulin was not observed within cellular or stromal elements of active or inactive Aschoff bodies either in endocardium or myocardium.

Except for the histochemical alterations described, specimens characterized by presence of bound gamma globulin did not exhibit any histopathologic changes not observed in specimens without gamma globulin. The comparative histopathologic findings in a group of 10 auricular biopsies with gamma globulin and in a selected group without such deposits, are presented in Table III.

TABLE III

Histopathologic Findings in Auricular Appendages in Relationship to Presence or Absence of Bound Gamma Globulin

No.	Patient	Bound gamma globulin			Histopathologic observations	
		Interstitial connective tissue	Myofibers	Vessel walls	Myocardium	Endocardium
1	San	++++	+++	++++	Considerable interstitial fibrosis and scarring of myocardium in some areas.	Slight to marked thickening. No focal aggregates of Aschoff cells. Small collections of lymphocytes in subendocardium.
2	Gas	++++	+++	+++	Several regions of old and recent interstitial fibrosis. Occasional Aschoff nodules in perivascular connective tissue.	Numerous large and small Aschoff nodules in irregularly thickened fibrosed endocardium and in septae projecting into myocardium. Within nodules, connective tissue edematous, collagen clumped and fragmented. Aschoff cells appear viable; many multinucleated.
3	Lau	+++	+++	+++	Scattered regions of increased interstitial connective tissue. A few small chronic inflammatory cell infiltrates.	Slight to moderate thickening. Few small collections of "inactive" Aschoff cells, with local clumping and fragmentation of collagen. Few giant cells.
4	Cap	+++	+++	+++	Little fibrosis of interstitial connective tissue. No cellular infiltrates.	Slight thickening. A single focus of chronic inflammatory cells.
5	Spi	+++	+++	++	Slight interstitial fibrosis. Muscle fiber degeneration evident. Few perivascular infiltrates of chronic inflammatory cells.	Little irregular thickening. No cellular infiltrates or Aschoff cells.
6	Shu	++	+	+	Increased interstitial connective tissue and edema. Rare Aschoff nodule in perivascular connective tissue.	Irregular fibrous thickening. "Active" Aschoff nodules, with numerous giant cells, degenerated fragmented, clumped collagen, edema, basophilic stroma. Several small aggregates of chronic inflammatory cells.
7	Hab	++	Traces	+	Few regions of perivascular fibrosis with mild chronic inflammatory cell infiltrates.	Irregular thickening and fibrosis and early degeneration of collagen. Small number of old "inactive" Aschoff cells.
8	DeA	+	+	+	Considerable perivascular fibrosis, and some myocardial fibrosis in subendocardial zone. No inflammatory cell infiltrates.	Moderate thickening and fibrosis. No focal cellular infiltrates or Aschoff cells.
9	Zam	+	+	+	Irregular fibrosis of interstitial connective tissue. Some perivascular chronic inflammatory cells.	Moderate fibrosis and thickening. Numerous aggregates of Aschoff cells in deeper endocardial layer, with edema, disruption, fusion, and clumping of collagen. Aschoff cells large and viable.
10	Urb	Traces	Traces	0	Irregular small regions of interstitial fibrosis.	Irregular thickening and fibrosis. Few "inactive" Aschoff cells. Small infiltrates of chronic inflammatory cells and mild edema.
11	Arc	0	0	0	Considerable interstitial fibrosis with chronic inflammatory infiltrates.	Little thickening. No Aschoff cells. Single focus of chronic inflammatory cell infiltrate.
12	Bar	0	0	0	Considerable increase in interstitial connective tissue, which appears edematous and basophilic.	Irregular thickening, with edematous, basophilic connective tissue. Small group "inactive" Aschoff cells. Aggregates of lymphocytes.

It will be noted that patchy myocardial fibrosis was a recurrent feature of the specimens with gamma globulin deposits. However, fibrosis in marked degree was also noted in some specimens without gamma globulin deposits.

Interstitial or perivascular infiltration by inflammatory cells, perivascular fibrosis, and mucoid degeneration of interstitial connective tissue were observed in hearts with and without bound gamma globulin. No correlation was evident between presence of bound gamma globulin and presence of Aschoff lesions, active or inactive, in endocardium or myocardium.

TABLE III—*Continued*

No.	Patient	Bound gamma globulin			Histopathologic observations	
		Interstitial connective tissue	Myofibers	Vessel walls	Myocardium	Endocardium
13	Wil	0	0	0	Focal regions of increased interstitial and perivascular connective tissue with chronic inflammatory cell infiltration. No Aschoff cells.	Moderate thickening and fibrosis. Groups of "active" Aschoff cells, small to large, multinucleated with fragmentation of collagen and edema, and basophilic staining of connective tissue.
14	Eil	0	0	0	Few small regions of interstitial fibrosis. No cellular infiltrates.	Irregular thickening. Many large and small aggregates of Aschoff cells with collagen fragmentation and degeneration, and edema. Aschoff cells large, multinucleated, "active."
15	Gra	0	0	0	Slight increase in interstitial connective tissue. Large Aschoff nodule containing large cells with amphophilic cytoplasm, fragmented edematous connective tissue. Few small infiltrates of chronic inflammatory cells.	Considerable thickening. No Aschoff nodules.

Attempts to evaluate in hematoxylin-eosin-stained sections altered myocardial sarcoplasm in the absence of inflammation proved unsatisfactory. Clumping or "solidification" of the sarcoplasm, with loss of cross-striation and with nuclear shrinkage was noted. Its significance could not be evaluated. This same difficulty was encountered by Enticknap (11).

DISCUSSION

The present report has described bound gamma globulin deposits within myofibers, sarcolemma, interstitial connective tissue, and vessel walls in a significant proportion of rheumatic auricular appendages. Neither albumin nor fibrin was associated with sites of such deposits. These sites also gave evidence of tissue alteration, as indicated by an enhanced affinity for eosin and a

strongly positive PAS reaction, and were frequently comparable in appearance to "fibrinoid" (13, 14). These findings were of particular interest because of the previously reported association of gamma globulin with fibrinoid in other lesions of the rheumatic disease group, such as systemic lupus (1, 3), rheumatoid arthritis (1), and periarteritis (2).

With respect to the possible relevance of these findings to rheumatic heart disease, myofibers, interstitial connective tissue, and vessel walls have all been shown to be involved in the pathology of this disease (15–17). Injury to myofibers, including focal waxy degeneration and necrosis has been frequently described (15, 18–25). Various aspects of the histopathology of myofibers in rheumatic heart disease and evidence that the "*myocardial* Aschoff body" may be derived from myofibers have been treated in detail by Murphy (25). The nature of the "*myocardial* Aschoff body" and its differentiation from the "classical Aschoff lesion" have been further considered by Saphir (12).

Diffuse and focal damage of interstitial connective tissue, including collagen and ground substance, with or without cellular reaction and proliferation, was described by Geipel (26) soon after Aschoff's (27) description of nodular proliferative changes, and was later extended by the work of Talalejew (28), Klinge (15), and others (9, 29, 30). The Aschoff body was considered by Klinge and by most subsequent authorities as a granulomatous reaction to altered fibrinoid collagen. The vascular changes in this disease comprise principally fibrinoid degeneration and necrotizing inflammation of small and medium sized vessels (18, 31–34).

The distribution of bound gamma globulin with associated tissue alteration within myofibers, interstitial connective tissue, and vessel walls in these rheumatic auricular appendages, thus appears consistent with a possible relationship to pathologic lesions of rheumatic heart disease. It will be noted, however, that bound gamma globulin was not observed in Aschoff lesions, either within the Aschoff cells or within the altered fragmented collagen associated with these lesions. Further, no correlation could be demonstrated between the presence of Aschoff lesions in endocardium or myocardium and presence of bound gamma globulin in these or other tissue sites. These results with washed unfixed sections are not directly comparable with the findings reported by Vazquez and Dixon (1) since the latter authors employed fixed tissue sections, in which gamma globulin associated with inflammatory exudate, normal gamma globulin, and bound material would all have been included in the specifically stained areas.

Association of gamma globulin, but not fibrin, with sites of eosinophilic alteration is consistent with previous observations of Dixon and Vazquez (35), that sites of fibrinoid in lesions of certain rheumatic diseases may show "increased concentration of gamma globulin with little or no evidence of an increase of albumin or fibrinogen." The absence of detectable fibrin or albumin

at sites of eosinophilic alteration in rheumatic auricular appendages excludes participation of these proteins in the observed tinctorial change. In a few of the auricular specimens examined, all three proteins, gamma globulin, albumin, and fibrin were found sparse or absent from sites of intense eosinophilic alteration. The data presented are consistent with the concept that an alteration of intrinsic tissue constituents rather than deposition of fibrin is the basis for enhanced affinity for eosin, and that this alteration, which is usually but not regularly associated with gamma globulin deposition, may affect myofibers, sarcolemma, and vessel walls, as well as collagenous connective tissue. In more recent studies, these same sites of alteration have been found intensely metachromatic with toluidine blue.

The central problem concerns the origin and function of gamma globulin in these rheumatic hearts and its relationship to the histochemical changes observed. The absence of other proteins, *i.e.* albumin or fibrin, in involved foci provokes consideration of a special function for the gamma globulin. Its localization in myofibers, sarcolemma, interstitial connective tissue, and vessel walls presupposes some mechanism of diffusion into and subsequent fixation in these sites. Since myocardial cells in various species studied have not been found permeable to either foreign or native serum proteins (36, 37), the basis for an altered myocardial cell permeability resulting in selective deposition of gamma globulin must also be sought.

Can these deposits of bound gamma globulin be reconciled with antigen-antibody reaction? If an autoimmune hypothesis be considered, fixation of gamma globulin might represent autoantibody deposited at the site of interaction with antigen, or deposits of autoantibody-antigen complexes fixed in tissues. However, limited validity can as yet be attached to this point of view, since the more basic question of the existence of autoantibodies to heart has remained unsettled. Both experimental and clinical aspects of this problem have been recently reviewed (38), and will be considered in further detail in the paper which follows.

The association of bound gamma globulin with "fibrinoid alteration" in rheumatic hearts is consistent with the participation of an immunologic mechanism, since fibrinoid changes may occur in lesions of serum sickness and drug hypersensitivity in man (39–42) and in tissue reactions of hypersensitivity in animals (43, 44). On the other hand, fibrinoid material has also been described in lesions not related to hypersensitivity (45).

From the non-immunologic point of view, the hypothesis that bound gamma globulin may be associated, in some way, with a special type of tissue response characteristic of "rheumatic inflammation," or that it may be attributable to tissue-reactivity of abnormal or complement-like globulins cannot be excluded. A non-specific binding of gamma globulin to altered tissue sites must also be considered, although this possibility seems weakened by the absence or sparse-

ness of gamma globulin noted in a few specimens with intense eosinophilic change. Correlation with myocardial fibrosis was perhaps suggested in the present study. Correlation with fibrosis as well as with fibrinoid was suggested also by the studies of Vazquez and Dixon (1).

One experimental approach to the characterization of bound gamma globulin considered from the immune point of view was to test the reactivity of rheumatic sera against normal and rheumatic heart tissue by an indirect immunofluorescent method (46) to determine whether serologic activity so observed might resemble the pattern of histologic distribution of bound gamma globulin in rheumatic hearts. This method of approach forms the subject of the following paper.

SUMMARY

Using fluorescent antibody methods, deposits of bound gamma globulin, as determined in unfixed washed sections of auricular appendages from rheumatic hearts, were noted in a significant number (18 per cent) of 100 specimens studied. Such deposits were observed in myofibers, sarcolemma, interstitial connective tissue, and vessel walls. Albumin and fibrin were generally found absent from these sites. Control hearts from normal and pathologic material, including postmortem and biopsied specimens, in general, did not reveal such deposits. These various tissue sites which contained bound gamma globulin frequently exhibited evidence of alteration as indicated both by enhanced affinity for eosin and by strongly positive reaction with the periodic acid–Schiff reagent, and appeared comparable in some cases to "fibrinoid." Bound gamma globulin was not observed in cellular or stromal components of Aschoff lesions, nor was the occurrence of Aschoff lesions correlated with presence of bound gamma globulin. It is suggested that deposition of gamma globulin and the eosinophilic alteration associated with such deposition are related to certain of the pathologic changes of rheumatic heart disease. The nature of such deposits of gamma globulin was considered from immune and non-immune points of view.

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EXPLANATION OF PLATES

All photomicrographs are from auricular biopsy specimens. Bound gamma globulin is visualized by brilliant yellow-green fluorescence in the ultraviolet microscope and is identified by the lightest areas in these black and white pictures, except as otherwise noted.

PLATE 1

FIG. 1. Auricular biopsy, patient For. Amorphous deposits of gamma globulin within myocardial cells (arrows). Neighboring cells as at lower right are devoid of material. Traces may be observed also in or adjacent to sarcolemma as well as in the interstitial connective tissue. × 400.

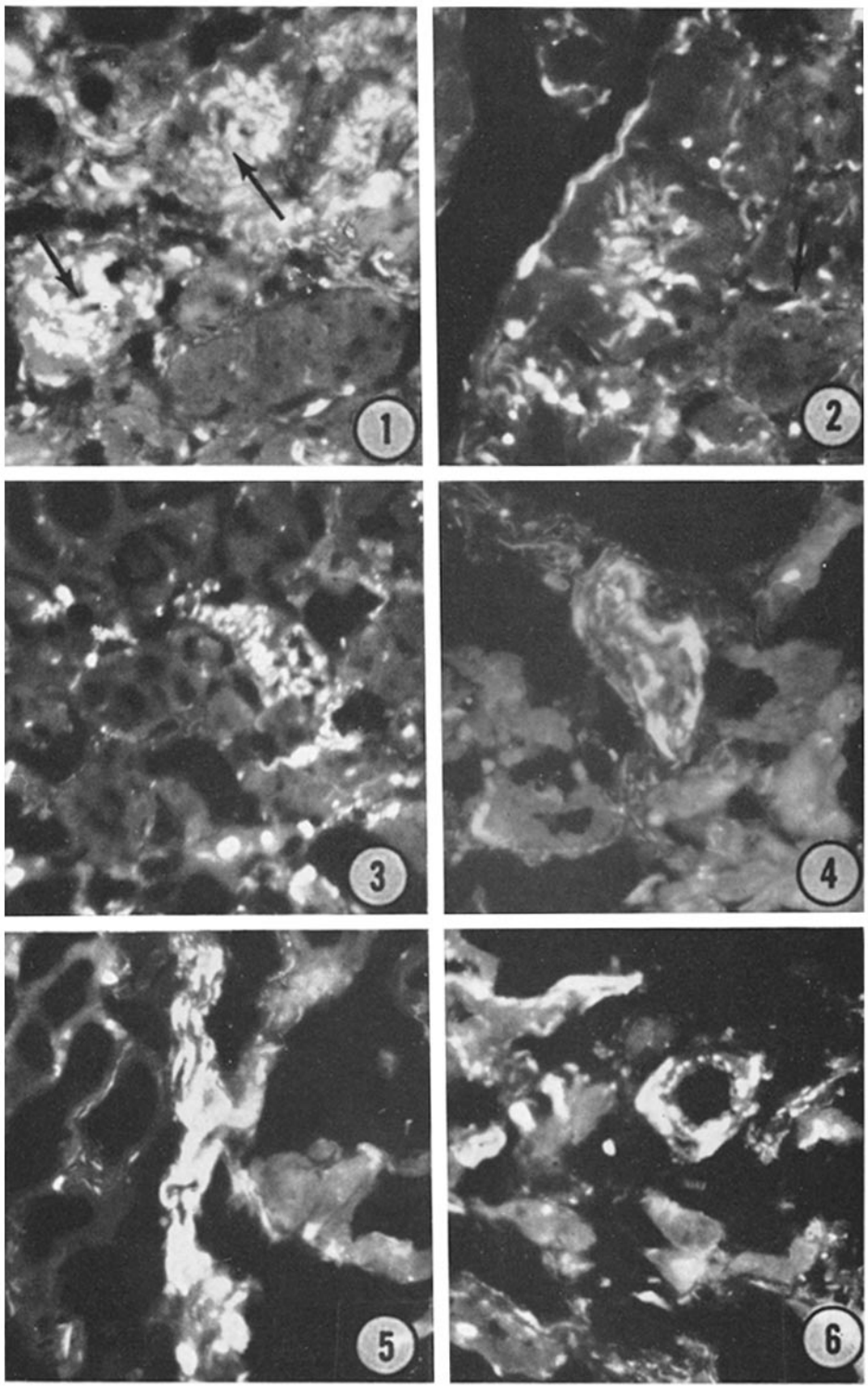
FIG. 2. Auricular biopsy, patient For. Gamma globulin is present within myocardial cells as rod-like condensations varying in thickness. Also, within these and in cells at upper right are delicate patterns of staining between myofibrils. Focal deposits are clearly demarcated within sarcolemma (arrow) and in the interstitial connective tissue. The small regular spherical bodies as at lower left are pink fluorescent pigment granules. × 400.

FIG. 3. Auricular biopsy, patient For. A myocardial cell with marked infiltration by gamma globulin stands in contrast from neighboring cells, of which one exhibits traces in sarcolemma. The gamma globulin in the cell occurs in rod-like distribution between myofibrils. × 400.

FIG. 4. Auricular biopsy, patient For. Gamma globulin extends from sarcolemma irregularly into the sarcoplasm of the cell. The cell at lower left exhibits specific staining in a segment of the periphery of the cell, including sarcolemma and subsarcolemma. × 400.

FIG. 5. Auricular biopsy, patient For. Diffuse and globular deposition of gamma globulin in wall of an arteriole. This vessel exhibited intense eosinophilic staining in parallel section stained with hematoxylin and eosin, and strong reaction with periodic acid–Schiff stain. × 400.

FIG. 6. Auricular biopsy, patient For. Segmental distribution of gamma globulin in the media and adventitia of a small artery. The internal elastic membrane is made visible by blue autofluorescence. The myofiber at upper left shows gamma globulin in peripheral sarcoplasm and sarcolemma. × 400.



(Kaplan and Dallenbach: Gamma globulin in auricular appendages)

PLATE 2

FIG. 7. Auricular biopsy, patient Hab. Focal deposits of gamma globulin in interstitial connective tissue frequently fused with segments of sarcolemma, and in some instances extending into the subsarcolemmal sarcoplasm. × 200.

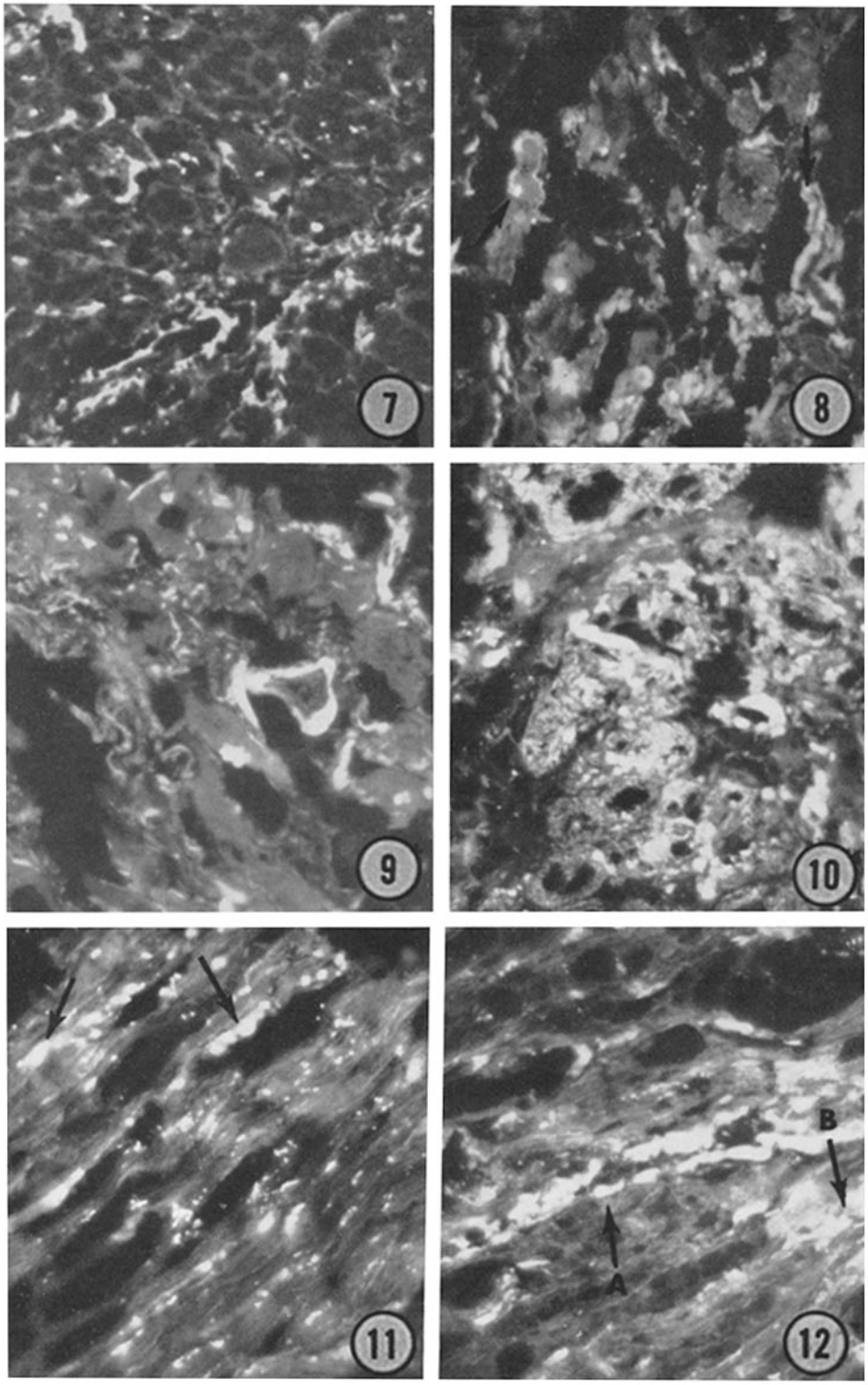
FIG. 8. Auricular biopsy, patient Hab. Sarcolemma and peripheral zones of myofibers (arrows) contain gamma globulin × 200.

FIG. 9. Auricular biopsy, patient For. Intense concentration of gamma globulin in peripheral portion of myofiber, penetrating sarcoplasm. × 400.

FIG. 10. Auricular biopsy, patient Lau. Extensive deposits of gamma globulin within a sector of auricular myocardium, with widespread penetration of myofiber sarcoplasm. Within the sarcoplasm, a delicate perimyofibrillar pattern of staining may be made out. × 400.

FIG. 11. Auricular biopsy, patient Spi. Linear globular deposits of gamma globulin along edge of myofiber and within myofiber sarcoplasm (arrows). The very tiny regular spherical granules scattered throughout the myofibers are pink fluorescent pigments. × 200.

FIG. 12. Auricular biopsy, patient Lau. Deposits of gamma globulin along the outer edge of myofibers, as at *A*, as well as within substance of sarcoplasm, as at *B*. × 200.



(Kaplan and Dallenbach: Gamma globulin in auricular appendages)

PLATE 3

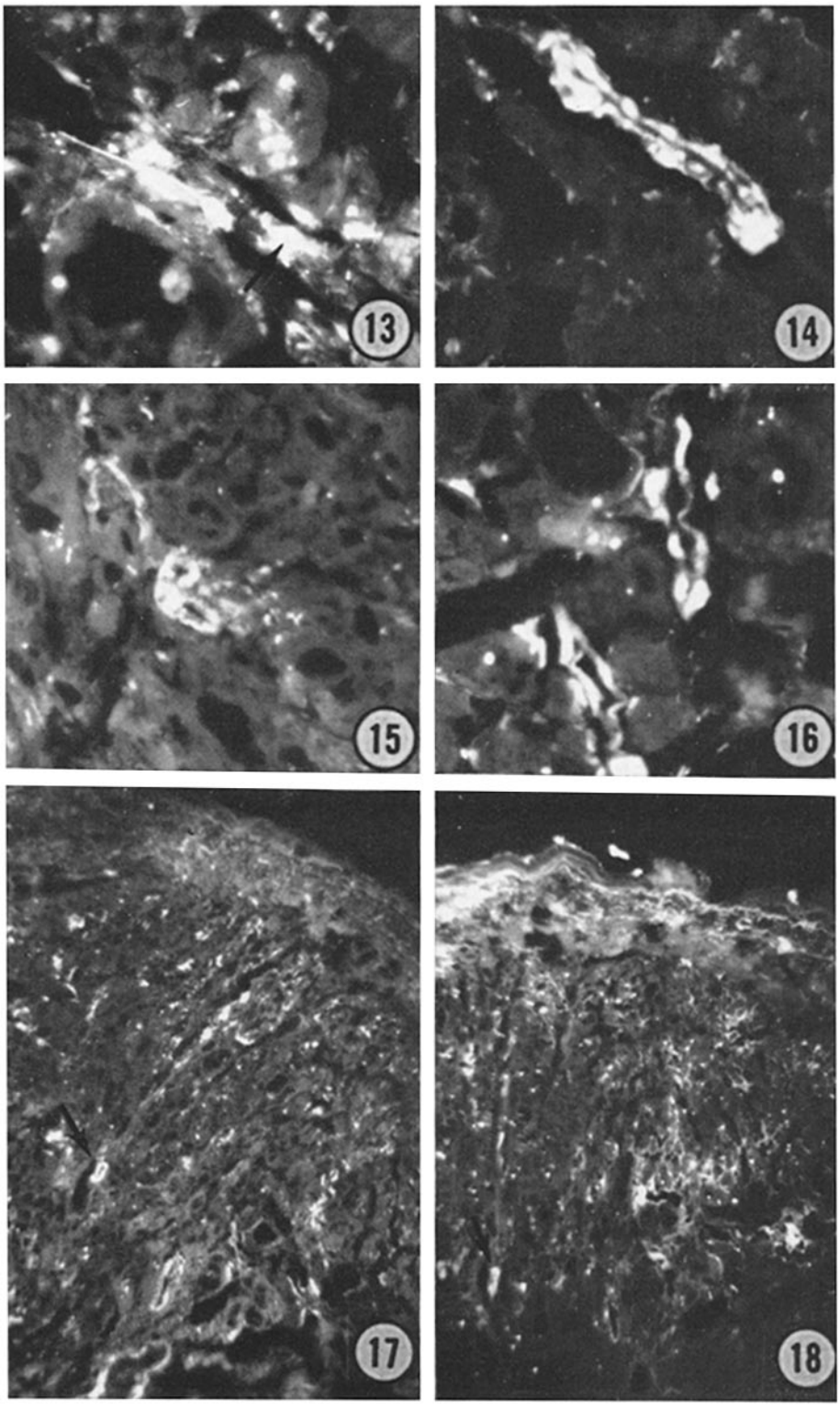
FIG. 13. Auricular biopsy, patient For. Deposits of gamma globulin within collagenous septum (arrow), with involvement also of an adjacent myofiber, above. × 400.

FIG. 14. Auricular biopsy, patient For. Gamma globulin deposits within wall of arteriole and traces in adjacent myofibers. × 400.

FIG. 15. Auricular biopsy, patient Lau. Deposition of gamma globulin in walls of branching arteriole. × 200.

FIG. 16. Auricular biopsy, patient For. Gamma globulin in walls of vessels, and in small focal segments of proximal myofibers on both sides of vessels. × 400.

FIGS. 17 and 18. Auricular biopsy, patient Lau. Comparative distribution of gamma globulin (Fig. 17) and fibrin (Fig. 18) in parallel but not adjacent sections. Vessel indicated by arrow may be used as reference landmark. Comparison of the distribution of these two proteins revealed no consistent relationship, although occasional overlapping of localization was noted in vessels and rarely in interstitial connective tissue. Fibrin is frequently present in the walls of vessels and in the interstitium of normal tissue sections. × 100.



(Kaplan and Dallenbach: Gamma globulin in auricular appendages)

PLATE 4

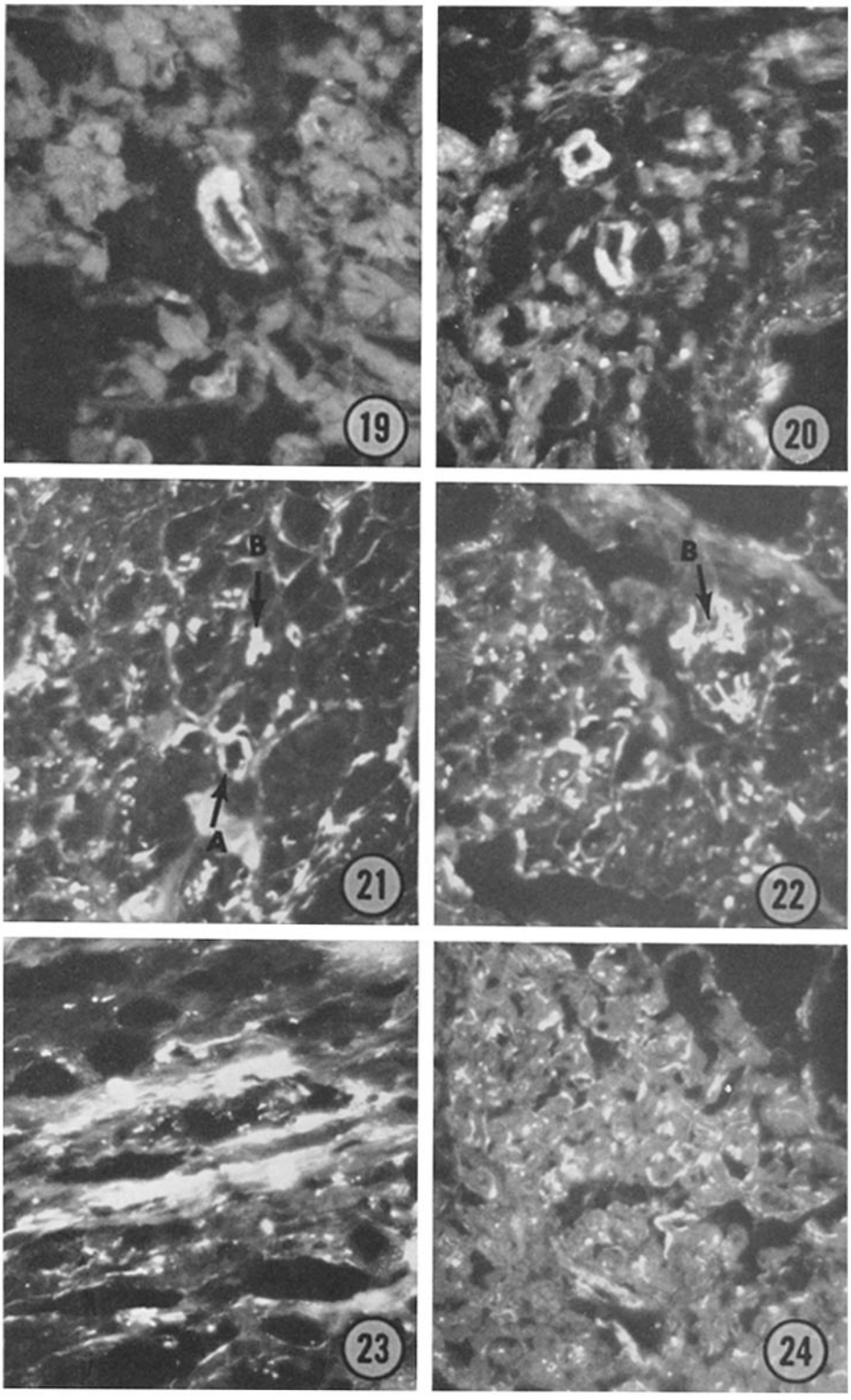
FIG. 19. Patient Gas. Gamma globulin in walls of arterioles × 200.

FIG. 20. Patient Gas. Eosin staining of arterioles by fluorochrome method. × 200.

FIGS. 21 and 22. Auricular biopsy, patient Lau. Affinity for eosin exhibited by interstitial connective tissue, sarcolemma, and vessel walls (*A*). Within myofibers, note staining of droplet-like condensations, clumps, and rod-like network, as shown at (*B*) in both pictures. Compare with gamma globulin distribution in same specimen shown in Figs. 12, 15, 17, and also with distribution of gamma globulin in specimen For shown in Figs. 1, 2. × 200.

FIG. 23. Auricular biopsy, patient Lau. Brilliant staining by eosin of focal condensations in sarcoplasm of myofibers, particularly in clumps and rod-like network, as well as in segments of sarcolemma. Longitudinal view. Compare with gamma globulin distribution in Figs. 10 and 12, from same patient. × 200.

FIG. 24. Auricular biopsy, patient Gas. Staining by eosin of segments of sarcolemma, peripheral portions of myofibers, and interstitial connective tissue. Distribution of gamma globulin in parallel section gave comparable pattern of staining. × 200.



(Kaplan and Dallenbach: Gamma globulin in auricular appendages)