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STUDIES ON THE PATHOGENESIS OF PULSELESS DISEASE

ESPECIALLY ON EXPERIMENTAL ANGITIS BY ELASTASE

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ABSTRACT

Twenty-three patients of pulseless disease were described with clinical and histopathological findings. Twenty-two cases whose ages range 16 to 41 were female and one was male. In nineteen of twenty-three cases surgical treatments were performed: removal of carotid body in 7, excision with Tetron graft in 4, by-pass prosthesis with Tetron in 5 and autogenous vein graft in 2 cases. Biopsies were taken in 13 cases, and the histological features were classified into 4 types: adventitis type in 8 cases, tuberculoid granuloma type in one case, giant cell arteritis type and diffuse productive inflammation type in two cases.

The main lesions were limitted to the media and the adventitia, and the intimal changes seemed to be secondary reaction, and the vasa vasorum may be primary sites of involvement of pulseless disease. The lesion of the elastic fibres seems to play an important role in the pathogenesis.

In order to clarify the exact etiologic relationship of primary elasticopathy to granulomatous reaction with elasticophagic giant cell, elastase suspension was infused in the common carotid artery of the rabbits intravascularly (37 cases) and extravascularly (21 cases), and elastolysis of the arterial wall was made, thereby, the elastogranulomous reaction and multinucleare giant cells phagocytosing the fragments of disintegrated internal elastic lamina were recognized in 2 cases (Figs. 15 and 16).

From the results of these experiments, it is clear that a causal relation exists between degenerating elastic fibres and granulomatous tissue reaction accompanying giant cell formation *in vivo*, and elastase has an elastolytic activity *in vivo*, and the fragments of the elastic fibres act as foreign bodies. The following conditions are necessary for the occurrence of elasticophagic giant cells: a specific destruction of the elastic fibres to provoke giant cells, for example, hard and slightly soluble fragments of the internal elastic lamina, and a mesenchymal activation brought about by some factors, one of which was typhoid-paratyphoid vaccination in this experiment.

The change in early stage of the elastic fibre by elastase was not observed *in vivo*, but at the incubation before elastolysis, metachromasia of the elastica became negative and no initial increase of the metachromatic reaction was observed. A newly formed elastic fibre was presented in early stage in the intimal thickening, but the regeneration of disintegrated elastic fibre was not seen in the

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media.

The effect of estrogen intensified metachromatic reaction of A.M.P. in the arterial walls, but it could not prevent the elastolytic action by elastase.

For the present, although exact etiology of pulseless disease is still unexplained, from clinical and experimental results, the elasticopathy associated with anti-elastin auto-immune mechanism or allergy following infection may be important in the pathogenesis of pulseless disease.

INTRODUCTION

Of the vascular disease in surgical field, it is known that pulseless disease of elastica type arteritis occurs mainly at the aorta and its main truncus of young women[1], and Buerger's disease of muscle type arteritis occurs mainly at the peripheral arteries of the four extremities of men[2].

Much problems are remained for the definition of pathogenesis of pulseless disease; for example, sexual specificity or relationship with the endocrine, localization of the lesion, infection, and allergy or auto-immune mechanism.

Histologically, the most characteristic histological features, so called meso-arteritis, are granulomatous inflammation and elasticophagic giant cell in the media.

From the histologic standpoint, elasticopathy seems to play an important role on the pathogenesis. The problem, whether this elasticopathy occurs primary or secondary, remains unkown.

For the solution of this problem, primary elasticopathy was produced by elastase administration in rabbits *in vivo* and essential conditions for the occurrence of the elasticophagic giant cells were found.

It is the purpose of this paper to describe twenty-three patients of pulseless disease, with particular reference to histopathological studies, and to clarify the operation of elasticopathy in the pathogenesis of pulseless disease.

CASE REPORTS

Tables 1, 2 and 3 show laboratory data, pulsation, blood pressure and chief complaint of pulseless disease in 23 cases respectively. The cases 2, 3, 4, 5, 10, 12 and 13 have already been reported[3].

SUMMARY OF CASE REPORTS

Twenty-three patients of pulseless disease were admitted to 1st department of surgery, Nagoya university school of medicine, between 1956 and 1966. The disorder encountered between the ages of 16 and 41, and 22 cases were female, only one was male (case 20). The age of onset of symptoms ranges from 5 to 41 years and most predominantly from 15 to 25 years old (52% of all present cases). Visual disturbances were seen in 16 cases: cataract in 5 cases, glaucoma in one case, and new growth and anastomosis of retinal vessels

in 7 cases.

On subjective complains, numbness and fatigue of upper extremities, particularly rapid exhaustion were present in 10 cases, local pain in 4 cases, headache in 4 cases and dizziness in 6 cases. Hyperactive carotid sinus reflex were present in 8 cases, and perforated septum nasi in one case.

On laboratory findings (Table 1), anemia was presented in 9 cases, and leucocytosis (more than 9000) in 8 cases, and increased erythrocyte sedimentation rate more than 20 mm were in 16 cases: the highest one was 98.3 mm at mean value. Total serum protein was between 6.4 and 9.3 mg/dl. Only two cases had abnormal electropholetic pattern which showed a mild elevation in γ-globulin fraction. Routine urinary tests were normal. Serologic test for syphilis were negative in all patients except case 7. Mantoux's reaction was positive except case 20 and serum choloesterol value was within normal limits. Seven cases had tuberculous anamnesis.

TABLE 1. Laboratory data in 23 patients

case	name, age	red cell count	white cell count	serum protein (mg/dl)	Al (mg/dl)	Gl (mg/dl)	A/G	chol. (mg/dl)	E.S.R. (mm)	Mantoux's reaction
1	K.O. 27	370×10^4	7200	8.5	—	—	—	—	58	?
2	S.M. 23	420×10^4	6200	7.0	3 6	2.6	1.1	—	43.3	+
3	S.S. 21	450×10^4	9800	7.0	3.4	3.6	0.9	—	58	+
4	U.T. 16	445×10^4	9600	7.7	5.1	5.0	1.0	144	98.3	+
5	H.K. 30	509×10^4	8000	7.8	4.6	3.2	1.4	129	14	+
6	U.I. 39	471×10^4	9200	7.0	4.1	2.9	1.4	129	33.5	?
7	M.O. 33	452×10^4	5400	6.9	2.8	4.1	0.7	198	24.3	+
8	Y.I. 16	405×10^4	8600	7.2	4.9	4.0	1.2	243	46.5	+
9	A.K. 18	390×10^4	6800	6.8	4.0	2.0	1.4	130	11.3	?
10	I.K. 27	334×10^4	4800	6.8	3.9	2.9	1.3	149	8.3	?
11	R.O. 29	400×10^4	7200	8.8	—	—	—	182	72.3	?
12	F.T. 32	484×10^4	8000	7.6	4.4	3.2	1.4	—	13.8	+
13	K.K. 33	310×10^4	10800	9.3	3.6	6.0	0.6	159	84.5	+
14	T.I. 36	370×10^4	9800	8.6	5.6	3.0	1.9	182	37.5	+
15	M.I. 41	380×10^4	9800	9.8	4.2	3.6	1.2	—	13.5	+
16	T.I. 16	383×10^4	5000	7.6	4.4	3.2	1.4	199	9	+
17	F.S. 19	447×10^4	9800	6.4	3.6	2.8	1.2	152	56	+
18	M.H. 26	446×10^4	11600	7.5	4.0	3.5	1.1	138	20.5	+
19	S.B. 41	398×10^4	9800	8.2	4.4	3.8	1.2	148	80.5	+
20	A.W. 20	502×10^4	8100	6.5	4.5	2.0	2.3	168	1.8	−
21	T.K. 20	380×10^4	5800	7.6	3.9	3.7	1.1	136	47.5	+
22	J.H. 20	330×10^4	4800	7.9	3.5	4.4	0.8	129	44	+
23	H.S. 37	295×10^4	6500	7.7	4.5	3.2	1.4	—	21	+

The brachial and radial pulsation were absent in 20 of 23 cases. No or decreased pulsation of the common carotid arteries was seen in 12 cases. Abnormal pulsation of the lower extremities were seen in 5 cases, among them 2 cases had intermittent claudication and one case had atypical aortic coarctation, but at operation aortic lesions were found in 5 cases. In these cases no significant difference was recognized between the right and left artery (Table 2).

TABLE 2. State of pulsations in 23 patients (thrill)

	case	carotid		axill.		brach.		rad.		fem.		popl.		post.ant. tib.		dor. ped.	
		r	*l*	*r*	*l*	*r*	*l*	*r*	*l*	*r*	*l*	*r*	*l*	*r*	*l*	*r*	*l*
1	K.O.	+	±	+	±	+	−	+	±	+	+	+	+	+	+	±	−
2	S.M.	±	±	±	±	−	−	−	−	+	+	+	+	+	+	+	+
3	S.S.	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+	+
4	U.T.	(+)	(+)	+	+	+	+	+	±	+	+	+	+	+	+	+	+
5	H.K.	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+
6	U.I.	+	−	+	+	±	±	±	±	+	+	+	+	+	+	+	+
7	M·O.	+	+	+	+	±	+	±	+	+	+	+	+	+	+	+	+
8	Y.I.	+	+	+	+	−	±	−	+	+	+	±	±	+	+	+	+
9	A.K.	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+	+
10	I.K.	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+
11	R.O.	+	±	+	+	+	+	+	±	+	+	+	+	+	+	+	+
12	F.T.	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
13	K.K.	−	−	+	+	+	+	−	−	+	+	+	+	+	+	+	+
14	T.I.	+	±	−	−	−	−	−	−	+	+	+	+	+	+	+	+
15	M.I.	+	+	+	+	+	−	±	−	+	+	+	+	+	+	+	+
16	T.I.	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+	+
17	F.S.	+	±	+	+	+	±	+	±	±	±	±	±	−	−	−	−
18	M.H.	−	±	−	−	−	+	−	−	+	+	+	+	+	+	+	+
19	S.B.	±	(+)	+	+	+	−	±	±	+	+	+	+	+	+	+	+
20	A.W.	+	+	−	+	−	±	−	±	+	+	+	+	+	+	+	+
21	T.K.	(±)	(±)	±	±	±	±	±	±	+	+	+	+	+	+	+	+
22	J.H.	(+)	(+)	+	+	−	+	+	−	+	+	+	+	+	+	+	+
23	H.S.	(+)	(+)	+	+	±	+	±	+	+	+	+	+	+	+	+	+

Arterial hypertension was found in 7 cases, and in 14 cases there was the difference of pressure more than 20 mmHg between paired arteries, and in 4 cases hypertension of the lower limbs was observed (Table 3).

Of 19 cases operated, removal carotid body was carried out in 7 cases, by-pass prosthesis with Tetron were in 5 cases, resection of the occluted vessel with Tetron graft insertion in 4 cases, autogenous venous graft in 2 cases and anastomosis of the carotid and subclavian arteries in one case. No surgical treatment was performed in 4 cases. Biopsies were taken in 13 cases, and the histological features were classified into 4 types as follows.

SURGICAL PROCEDURES AND LONG TERM BEHABIOUR

The follow-up results in 19 of 23 cases (about 83%) from 2 months to 10 years postoperatively were as follows (Tables 4-7). The results are rather poor. As for the dizziness, removal of carotid body was effective, and good or fair results were obtained in about a half of the cases. As to eye symptoms, only one (case 21) has obtained the improvement of vision.

The result of reconstructive surgical procedures in pulseless disease is worse than those in arteriosclerosis, for the involvements of pulseless disease were widespread and not segmental[4)5)].

These 19 patients are still alive, and 11 patients are spending useful live. However, in remaining 8 cases, their lives are restricted mainly by visual

TABLE 3. Blood pressure and chief complaint of 23 patients

case		blood pressure (mmHg)				chief complaint
		arm		foot		
		right	left	right	left	
1	K.O.	220/124	150/ 0	—	—	dizziness
2	S.M.	—	—	—	—	visual disturbance
3	S.S.	—	—	160/ 88	74/ 66	dizziness
4	U.T.	180/ 52	98/ 50	—	—	pain of the neck
5	H.K.	—	124/ 70	—	—	tired of the *r*-arm
6	U.I.	102/ 90	—	—	—	numbness of the *l*-arm
7	M.O.	90/ 76	114/ 70	—	—	pain of the *r*-arm
8	Y.I.	94/ 68	118/ 64	—	—	headache
9	A.K.	—	—	92/ 78	110/ 0	visual disturbance
10	I.T.	—	118/ 70	—	—	numbness of the *r*-arm
11	R.O.	158/ 0	80/ 66	—	—	dizziness
12	F.T.	92/ 42	82/ 60	—	—	headache
13	K.K.	150/ 90	—	—	—	dull pain of the sholder
14	T.I.	—	—	170/ 90	—	visual disturbance
15	M.I.	116/ 84	—	—	—	dizziness
16	T.I.	—	120/ 80	—	—	numbness of the *r*-arm
17	F.S.	150/ 78	116/ 96	—	—	intermittent claudication
18	M.H.	—	—	140/ 80	—	visual disturbance
19	S.B.	120/P	120/P	—	—	numbness of the *l*-arm
20	A.W.	80/ 60	100/ 70	—	—	tired of the *r*-arm
21	T.K.	—	—	130/ 60	140/ 60	numbness of the *r*-arm
22	J.H.	150/ 60	—	—	158/110	pain and tired of the *l*-arm
23	H.S.	114/ 90	140/ 80	214/110	210/120	numbness of the *r*-arm

TABLE 4. On the results of removal of carotid body

	case	follow up	result		
			good	no change	bad
unilateral	2	1	1	0	0
bilateral	5	5	2	2	1
not operated	16	13	0	6	7

TABLE 5. On the syncopal attack

	case	follow up	result		
			good	no change	bad
carotid body denervation	7	6	3	3	0
no denervation	16	13	0	6	7

TABLE 6. On the headache

	case	follow up	result		
			good	no change	bad
carotid body denervation	7	6	2	2	2
reconstructive surgery	7	7	1	3	3
other treatment or not treat.	9	6	1	1	4

TABLE 7. Follow up results after the reconstructive surgery

method	case	follow up	result		
			good	no change	bad
transplantation with Tetron graft	8	7	1	4	2
thromboendarterectomy	2	2	0	1	1
by-pass grafting with auto-vein	2	2	0	2	0
anastomosis of carot. and subcl. a.	1	0	0	0	0
biopsy	3	3	0	3	0
carotid body denervation	3	2	2	0	0
not operated	4	3	0	2	0

disturbance.

These results will be and should be improved with more treatment in early stage.

SUMMARY OF PATHOLOGIC FEATURES

In thirteen of twenty-three cases, biopsies were performed at operation and the histological features are classified in 4 types as follows:

1) Adventitis type; the pathologic changes are mainly localized in the adventitia. There are remarkable fibrosis and occlusion of the vasa vasorum in the adventitia, and degeneration or atrophy of the muscle fibres is marked in the outer layer of the media, and the elastic fibres are relatively preserved, therefore the media appears to be constituted by the elastica alone. The intima shows remarkable fibrous thickening with new formation of the elastica. Metachromatic reaction is positive only in the intima. In case 21, the thickened intima of ascending aorta has fibrinoid degeration at the luminal portion (Figs. 1–5) (cases 2, 12, 16, 17, 18, 21, 22 and 23). In active lesions, there is infiltration of inflammatory cells around the vasa vasorum in the adventitia (case 22: biopsy was taken 2 months after the onset).

2) Tuberculoid granulomatous inflammation type: destroy and tuberculoid granuloma in the media are main findings, and there is noticeable infiltration of plasma cell and lymphocyte around the vasa vasorum in the adventitia. Although muscle fibres disappear in the center of tuberculoid granuloma, the elastic fibres remain surrounded by the epitheloid cells and elasticophagic giant cells. The intima has edematous thickening with remarkable proliferation of young connective tissue cells. Only the intima shows positive metachromatic reaction (Figs. 6 and 7) (case 7).

3) Giant-cell arteritis type: elastolysis and granulomatous formation with elasticophagic giant cell are the striking changes, and there is a remarkable fibrosis in the adventitia. The media is destroyed and replaced by granuloma with elasticophagic giant cells, and by focal or diffuse collections of lymphocytes, plasma cells and neutrophilic leucocytes. These histologic features resemble those seen in giant cell arteritis.

The intima has marked edematous thickening with remarkable formation of new vessels and acid mucopolysaccharide (AMP) rich substance (Figs. 8 and 9) (cases 5 and 13).

4) Diffuse productive inflammation type: panarteritis as seen in Buerger's disease is the characteristic findings, and there are extensive fibroblastic proliferation and foci of inflammatory cells in the adventitia, and diffuse infiltration of round cells and foci of destroy of the elastica with elasticophagic giant cells in the media. The lumen is completely occluded by the intimal thickening and organized thrombus. Only intima shows positive metachromatic reaction (Figs. 10 and 11) (cass 10 and 20).

From these histologic features, pathogenesis of pulseless disease are suggested: 1) adventitial origin, 2) the relationship with tuberculosis, 3) identity with giant cell arteritis, 4) the similarity to Buerger's disease. However, it is impossible to decide whether these four categories imply multiple etiological agents or indicate various features of the same disease in different stages.

The main lesions were limitted to the media and the adventitia, and the intimal changes seemed to be secondary reaction, and the vasa vasorum may be primary sites of involvement of pulseless diesease. The lesion of the elastic fibres seems to play an important role on the pathogenesis of pulseless disease. However, the histogenesis of granulomatous reaction with giant cell to disintegrated elastic fibres is unknown.

The present experiments were carried out to determine whether a cause-and effect relationship exists between degenerating elastic fibres and granulomatous tissue reaction accompanying giant cell formation *in vivo*.

MATERIALS AND METHODS

Rabbits weighing 2.5 to 3.0 kg were used. In order to destroy elastic fibres of the vessel walls, "elastase" suspension (0.3, 0.5 and 1.0% N.B.C. and Sigma Co. U.S.A.) with 0.2 M borate buffer at pH 9.0 was administrated to the common carotid or femoral arteries.

Elastase was given by intravascular infusion under firmly applied double ligations (Exp. A) or periadventitial injection (Exp. B). Also an attempt was made to activate the reticulo-endotherial system by typhoid-paratyphoid vaccine, which was administrated intraperitoneally in a dose of 3 ml per kg initially followed by a daily dose of 0.5 ml to the neck skin or 3 ml to the intraperitoneal cavity for a few days to 14 days.

With the same attempt the B.C.G. vaccine was administrated intravascularly initialy in a dose of 50 mg per kg, and a second dose of 50 mg to the peri-carotid tissues after 30 days (4 rabbits).

Experiment A; After submitting the vessels to the action of elastase for 20 minute to 7 days before the ligatures were released, the segments of the

vessels were removed at intervals ranging 2 to 90 days (37 rabbits).

Experiment B: The segments of the vessels were removed at intervals ranging from 2 to 30 days (21 rabbits).

Experiment C; After submitting the common carotid arteries to the action of elastase (as in expts. A and B) four young rabbits aged 3 weeks, 3 males and a female were treated as follows: A 10 mg of estrogen (estradiol succinate 20 mg Organon Co. Holland) was administrated daily subcutaneousely for 7 to 21 days, and biopsies were taken weekly for 4 weeks.

Experiment D; 0.3% trypsine solution with 0.1-M veronal buffer at pH 8.0 was administrated intravascularly in the common carotid arteries, as done in experiment A. And only borate buffer at pH 9.0 was administrated similarly as control.

Experiment E; The common carotid arteries had been doublely ligated with some blood remained between two ligatures. The segments were removed weekly for 14 weeks.

Experiment F; The films of vessel walls were incubated in 0.1% elastase suspension and 0.1% trypsine solution at 10 minute intervals for 60 minute.

Routinely the removed vessels were fixed in Carnoy's solution for 2 hours, then embedded in paraffin. Sections at 6 μ were prepared. Various staining were used as follows; 1) hematoxylin and eosin, 2) Weigert's resorcin fuchsin and van Gieson stain, 3) toluidin blue stain by Oono's modification (at pH 2.5, 4.1 and 7.0)[6], 4) periodic acid Schiff's reaction (PAS reaction), 5) Azan Mallory stain, 6) phosphotangustenic acid hemtoxylin (PTAH), 7) Pap's silver impregnation.

In the experiment C, histochemical examination of A.M.P. in the films of vessel walls were also done according to the following method; 1) hyaluronidase digestion, 2) methylation (treatment with conc. HCl 0.8 ml methanol 100 ml for 2 hours at 58°C), 3) methylation-demethylation (immersion in 0.1% KOH solution in 80% alcohol for 20 minute at room temp.) sequence. 4) acetylation (treatment with 40% acetic anhydrite in pyridine for 24 hours at 37°C) and acetylation-deacetylation (collodinization and incubation in 20% NH_4OH solution in 90% ethanol for 24 hours at 37°C) sequence. 5) all films were stained histochemically, that is, stained by toluidin blue dye at pH 7.0 and 6) Alcian blue stain (0.005% Alcian blue in 1.0% acetic acid solution at pH 3.0) sequence.

EXPERIMENTAL RESULTS

Relationship between elastase concentration and elastolysis in the vessel wall: Elastolytic activities were too weak in 0.1% elastase suspension, however, in 1.0% elastase they were too noticeable to investigate the degenerative process of elastic fibres. There is no different elastolytic activity between 0.3 and 0.5% suspension.

Experiment A

Although no noticeable degradation of elastic fibres were seen during the first 20 minute of attack by elastase, remarkable elastolysis was recognized after 40 minute.

Changes in the intima;

One week after the injury, the lumen was occluded by thrombus and hyperplasia of the intima in 10 cases, no thrombosis was found except in one case, and blood stream was seen in 10 cases. There was a concentric or eccentric thickening of the intima, and the intimal thickening composed with mucopolysaccharide rich ground substance, fibroblast, newly formed reticular fibres and granular elastica stainable material,—elastoidosis—. The extent of thickening corresponded to the degree of damage to the underlying media.

Two weeks after the injury smooth muscle cells were present and elastoidosis are changed to fine fibrous materials—elastosis—.

Four to five weeks after the injury the elastosis was darkly stainable by elastica stain, the intimal thickening became remarkable. At the long term observation the thickened intima was composed of smooth muscle cells, elastic fibres and reticular fibres. Luminal portion of the intima or inner parts of newly formed vessels were covered with the formation of newly endothelial cells. However, the elastosis is finer than the fibrous element itself. Morphology of the elastosis is different from that of original elastica (Fig. 18).

Changes of the internal elastic lamina;

Disorganization and fragmentation of the elastica were recognized. Fibrinoid degeneration (blue by PTAH, red by Azan-Mallory stain and yellow by Weigert's resorcin fuchsin and van Gieson) by hyper-permeability of the endothel was occasionally observed.

Three to four weeks after injury there was a noticeable thickening of the intima, which usually occurs on the affected part of the internal elastic lamina. The boundary between the intima and media became obscure. Degradated internal elastic lamina is remained as a fragment even after 3 months but regeneration or reconstruction was not seen (Fig. 18). On the other hand, the stainable materials by elastica dyeing were seen ring likely at luminal region of the thickening intima.

Changes of the media;

A week after the injury there was a remarkable destroy of the elastic fibres and degeneration of the smooth muscle cells. Three weeks after the injury destroyed portion of the media was compensated with a thickening intima (Fig. 17).

Changes of the adventitia;

In all cases there was prolyferation of fibroblasts and mononuclear cells,

and no infiltration of neutrophilic leucocytes were seen (Fig. 16).

On metachromatic reaction;

In the very early stage of within 48 hrs, in all layers a orthochromasia was seen, more than one weeks after the injury the intimal thickening showed positive metachromatic reaction. In cases of thrombotic occlusion a metachromasia was seen around newly formed vessels and internal elastic lamina remained in early stage. The media became orthochromatic by elastolysis, but after two weeks it was stained as metachromatic.

Mutinucleated giant cells phagocytosing the fragments of disintegrated internal elastic lamina were recognized. In these cases the vaccine are injected at pre- and post-injury, and neither neutrophilic leucocytes nor foreign body was presented (Figs. 15 and 16) and in the cases without injection of the T.A. vaccine, an intense elastolysis and a patency were seen, but there was no giant cell reaction and no noticeable inflammatory cell infiltration.

In the cases with injection of the B.C.G. vaccine, a noticeable perivascular cell infiltration with foreign body giant cells are seen, there was neither thrombus nor elastogranuloma cell reaction.

Experiment B

In all cases no thrombus was seen, at the immediate stage—within 48 hrs —, disintegrated elastic fibre was seen in the media. Three to four weeks after the injury eccentric thickening of the intima occurred on only the affected part of the media, in the widespread cases of elastolysis intimal thickening was seen in the girth. In the cases of intense degradated elastic fibres a fibrinoid degeneration and aneurysm formation were seen. Ten days after the injury, brilliant cells were presented, that were derived from the smooth muscle cells. The destroyed regions in the media are compensated by the intimal thickening. Irrespective of the vaccination neither elasticophagic giant cell nor thrombus was presented, although an intimal thickening as well as destruction of the media and adventitia was seen (Figs. 19, 20 and 21).

Although within 7 days after the injury, metachromatic reaction was not present in elastolytic regions, and after 2-3 weeks thickened intima and degenerated media were metachromatic, but such metachromatic material had no relationship with the elastic fibres.

As for the control borate buffer was infused, but no change was observed in the vessel wall (Fig. 23).

Experiment C

Sexual specificity which is influenced by estrogen was not observed. Two to three weeks after the intimal hypertrophy and thrombotic occlusion were seen, but there was no difference of intimal thickening between the cases with and without estrogen infusion. In the thickened intima, metachromatic reaction,

PAS, Alcian blue were positive. The metachromatic material was resulted in histochemically as follows: Methylation diminished to a variable extent metachromatic stainability and this suppressive effect was largely unaltered by subsequent demethylation. Acetylation reduced significantly or abolished the metachromatic staining of the films, which tended, however, to be recovered by subsequent deacetylation. Estrogen have no protective effect to disintegration of elastic fibres and hyperplastic effect of the intima.

In all cases with vaccination, elastogranuloma reaction without giant cell reaction was seen.

Metachromatic material of case 21 was more resistant for acetylation of the film than case 22.

Experiment D

One week after the infusion of trypsin, although no degradation of elastic fibre was seen, remarkable degeneration and disappearence of the muscle fibres with intimal hypertrophy and fibrinoid degeneration in the subendothelium were recognized. Two weeks after the infusion the lumen was occluded with thrombus and intimal thickening. Because the muscle fibre in the media had disappeared, the media was constituted of the elastic fibre alone. There was no red stainability by Azan Mallory stain and no blue stainability by PTAH in the media. And still more after three weeks the elastic fibres were almost preserved. Remarkable intimal hypertrophy with elastosis and newly formed muscle fibres were seen (Fig. 22).

In innerportion of the media and intima thickening, metachromatic reaction was positive.

Experiment E

One week after the double ligation blood was seen in the lumen, and the clot was constituted mainly by thrombocyte and leucocyte, but no organization was seen. A part of the endothel had the desquamation, and the internal elastic lamina, media and adventitia remained intact.

Two weeks after the ligature the organization of the clot in the lumen began, intimal hypertrophy was seen with the elastoidosis in the midsegement and the thickening of the intima, while the thickening of the intima was ring-like distinctly and frequently more marked on one side, but media and adventitia were intact.

Three weeks after the ligature intimal hypertrophy became more even along the vessel wall and more remarkably, that is constituted of the fibroblasts and round cell. The media had no changes, however, the vasa vasorum in the adventitia dilatated.

Four weeks after the ligature intimal hypertrophy became more intensified, thereby the original lumen was completely obliterated, in such as a part is filled by the organized thrombus with recanalisation. In the intima thicken-

ing newly formed muscle cell appeared, and elastosis became distinct.

Five weeks after the ligature the elastic and muscle fibres in the media were disintegrated and disturbance of the disposition were seen. The border between the thickening intima and the media became obscure, the intima is occupied mostly by young connective tissue cells. The communication by the newly formed vessels between the vasa vasorum and the original lumen was seen.

Twenty weeks after the ligature the lumen was entirely obliterated with remarkable recanalisation. The elastica in the media was markedly disintegrated, but it remained as fragment.

Experiment F

The films of the vessels were incubated by 0.1% elastase suspension, in an onset of the incubation or after 10 minute no elastolysis of the arterial walls occurred, but metachromasia which is derived from the elastic fibres changed to orthochromatic.

In the case of 20 minute incubation the internal elastic lamina was intact, but the elastica of the most inner part became fragments, and PAS reaction became negative (Fig. 12).

In the case of 30 minute incubation the destroy of the elastica developed to the deep layer, and in the case of 60 minute, the most elastica are remarkably destroyed, therefore, these elastica remained only a trace. Acidphilic property of the muscle cells were seen feebly, however, the morphological chanse was not seen (Fig. 13).

After 60 minute incubation by 0.1% trypsin solution, the elastic fibre remained intact, but after 20 minute incubation acid philic property of the muscle fibre was lost, and with time the morphologic change occurred (Fig. 14).

These changes of the muscle cells were seen not only in the vessel walls, but in the muscle cells around vessel walls. Ten minute after incubation metachromatic and PAS positive reaction became already negative.

The changes of femoral artery and jugural vein by elastase were too noticeable to investigate the degenerative process of the vessel walls. That is, an intense destruction in pan-arterial wall was seen, and neither elastogranuloma reaction nor regenerative process was seen.

DISCUSSION

The elastic fibre in the arterial wall

The elastic fibre is one component of the vessel wall, however, the early chemical studies on the composition of the elastic tissue were greatly hampered by three major difficulties: 1) the elastic fibres everywhere are associated with collagenous and reticular fibres, and the separation was difficult. 2) insolubility and lack of reactivity except affinity for certain selective stain. 3)

elastin from different sources and even from the same source at different ages, has different compositions[7]. But since elastase was discovered, rapid advanced observations of elastic fibres have been taken[8)10].

As to the structure of the elastic fibrillar core surrounded by a carbohydrate-rich protein sheath which is mucopolysaccharide protein complex[12].

Pathologic degeneration of the internal elastic lamina begins by duplication *in vivo*, and those changes are influenced by age and grade of the intimal thickening[18]. In the present experiment the elastic fibre in the media is digested by elastase, consequently, the disarrangement of the smooth muscle cells and transformation to brilliant cell are seen (expt. B). Such a brilliant cell is one of wandering cells and in human body, it exists normally, but not in the rabbit[19].

Experimentally, intimal thickening occurs in 2 weeks after the injury and in 7–12 weeks after the lumen is completely occluded[20)21]. In the present experiment (E) the occlusion of lumen is seen within 4–5 weeks. On the other hand, pathologic occlusion of vessels begins in 5 days after the attack[22]. Elastic stainable material which is seen in thickened intima is observed as grobular in 2 weeks after, —elastoidosis—, and it is showed as the fibrous structure at 3 weeks after the injury. After a long term observation, the net work in the thickened intima is observed—elastosis—(Fig. 15).

In the present experiment (D), the degeneration and the disappearance of the smooth muscle cell in the media occurred by trypsin infusion, but, thickened intima was not observed (Fig. 22).

Kunz and Goder (1963)[25] had tried the differentation between new elastosis or elastoidosis and original elastic fibre. In the present experiment, in the case of long-term observation, new elastic fibre was observed in the thickened intima, but the regeneration of the elastic fibre in disintegrated media was not found (Fig. 18).

Acid mucopolysaccharide in the elastic fibre

Acid mucopolysaccharide (AMP) is a structural material in the arterial walls, and it exists to the end of life[11)26]. In the intima, AMP exists until fibrous lesions become to be degenerated and hyalinized. Generally, it is known that AMP exists most abundantly in the inner one third of the aortic media, a little in the middle and very slightly in the outer one third portions[26]. AMP exists already in fetal stage, and it increases after birth and such tendency continues until the adolescence. It becomes constant at thirties or fourties, but after forties it decreases slowly with the development of fibrosis and diminution of the elastic fibre is also seen[27].

AMP exists not only as a component of the matrix, but it relates with the repair in pathologic vessels[27)30], and has anticoagulant activity[28]. According to Wexler *et al.* (1964)[32], aortic medial mucopolysaccharide increases in its

concentration with progression on arteriosclerosis. Also, the accumulation of AMP is observed at some stage in rheumatic disease and an early stage in fibrinoid formation[30].

Likar *et al.* (1965)[33] discussed on sexual cycle and AMP levels in the aorta which respond to hormones regulating the sexual cycle. And it may be one of the reasons that premenopausal women are resistant to atherosclerosis.

Estrogen acts to high polymerisation on AMP in the matrix, thereby sol-gel balance is shifted toward the gel side *in vivo*, thence estrogen acts as hemostathicus, under mechanism that the vessel wall shows resistance for the permeability[34]. This increased material—highly metachromatic material—is digested by hyaluronidase, and it undergoes methylation, therefore it is obviously an acid compound having COOH or SO_3H groups, one of which at least may be hyaluronic acid[34]. However, it is a problem to be elucidated that the metachromatic material in the media of pulseless disease is hyaluronic acid[35], because the film of the vessel is stained in Alcian blue and it responses to acetylation (expt. C). However, one of them, AMP, may be a hyaluronic acid which contains COOH group.

Also, highly polymerized AMP by estrogen infusion in the vessel wall is easily depolymerized by elastase, but the reaction as elastase inhibitor is not observed. The occurrence of AMP other than hyaluronic acid is not proved, however, it may be taken plase by the same course as hyaluronic acid[13]. In the course where the elastic fibre taken in protoplasm of giant cell is digested, the release of AMP in the elastic fibre is observed, but in the present experiment the relationship between AMP of the elastic fibre itself and elasticophagic giant cell was not elucidated. Experimentally, Kunz (1962)[14] confirmed that the elastogranuloma was formed when the elastic substance of the human aortic media was transplanted to the subcutaneous of rat, and the non-specific biosynthesic action of neutro- and acid-mucopolysaccharide was observed in the intercellular space of that elastogranuloma.

On the elastic fibre which is incubated in elastase, the following result was observed: an early changes, the glassy refractivity of the elastic fibre was lost[7][10] and the increase of metachromatic material was seen[29]. These main changes are characterized by swelling of the elastic fibre and desquamation from over the elastic fibre[37]. In the present experiment (B), only orthochromatic reaction was seen in the site where elastase was injected. On the other hand, in the present experiment (A), in spite of the fact that the elastic fibre in the media was remarkably destroyed, metachromatic reaction was seen. On this point, three causes are presumed as follows: 1) AMP of the elastic fibre itself is digested by elastase, yet, AMP in matrix remains, 2) on the surface of the elastic fibre in the injured vessel wall, the disturbance of polysaccharide metabolism is provoked. 3) the fibroblast which occurs as reconstructive course of the vessel wall may originate[31].

The change of metachromatic reaction of AMP is not observed *in vivo*. Also, in the present experiment (C), the same results as in experiment A and B is obtained *in vivo*. From these results it can be supposed that AMP is depolymerized by elastase action in an early stage. In fact, neither the isolation of AMP nor increasing of metachromatic materials was seen.

Action of elastase on the elastic tissue

The presence of an enzyme in animal pancreatic extracts which solubilized elastin was first described by Balo and Banga[8]. It is so called elastase, and it has the specific elastolytic property, the reaction of which is seen on only the elastic fibre, but not on collagen[9][10]. Since then, many studies have been taken on the structure of the elastic fibre and on the pathogenesis in arterial disease[38].

Balo and Banga (1949)[9] also discovered that elastase inhibitor exists in human, rabbit and ox serum and pancreas. Since then, the relationship between arteriosclerosis and volume of elastase inhibitor have been investigated, but it's problem is not put aside[37].

Balo and Banga (1954)[29], Sölyom (1964)[39] reported on early changes of the elastic fibre by elastase, that initially swelling of a piece of arterial wall and nuchal ligament occurred, and secondary vacuolization of the elastic fibre of the vessels, while elastic tissue of nuchal ligament broke up into perpendicular fractures, and at last these elastic tissues dissolved quickly. In the present experiment these phenomena were not seen. Elastase may have two active factors, these names are E-1 and E-2: E-1 to loose the polysaccharide, and E-2 to break the links between polysaccharide and the protein.

Banga (1953)[40] said that the action of elastase is not proteolysis, but depolymerisation of protein molecular. Hall (1952)[16] said that effect of elastase reacted as no protolytic action, but as mucase, and it reacted only to elastomucin chain, then proelastin was isolated. In the present experiment elastolysis by elastase is proved *in vivo*, and it is supported that initial action of elastase is depolymerisation of AMP because at early stage by elastase incubation metachromatic reaction changed to orthochromatic (Figs. 12 and 13) (expt. F).

According to Hall (1952)[16], when proteinase such as trypsin acted on the elastic fibre for a long time, it acts on proelastin in the elastic fibre, and as the result the elastic fibre is digested. However, in the present experiment (C) with the vessel wall to which trypsin was infused, and early change was not seen while two and three weeks after the injection change of atrophy or degeneration in the media was seen, these observations agree with Ikenaka's results[43].

The giant cell reaction

The giant cell formation under the physiologic and pathologic condition have various types. It was known that Langhans's cell type, foreign body

type, atypical Langhans's cell type and myogenous giant cell occur in elastica type arteritis. The elasticophage, so called foreign body type giant cell is of the most generality, while Langhans's cell type may be little significant[23]. So called temporal arteritis[44] or giant cell arteritis[45] is a specific granulomatous arteritis, characterized by a peculiar destruction of the elastic fibres which results in giant cell reaction, but the presence of giant cells in giant cell arteritis is not considered to be of great significance[45][46]. In some cases of Buerger's disease giant cell is seen, therefore, the elasticophage is not exclusive feature[48].

Since Leo-Rau (1932)[49] many studies have been taken, in which giant cells are mainly formed by fusion of large type mononuclear cell and/or histiocyte. The histiocyte is one type of the phagocytic cells that occurs by activation of reticulo-endothelial system[50]. The formation of foreign body type giant cell is related not only with the mesodermal cell, but many ecto- and endodermal cell, also, occurs rarely from leucocyte and wander cell[45].

It remains to be defined whether proliferation of giant cell depend on mitosis or amitosis, but it is demonstrated that no mitosis is seen in giant cell, and generally, fusion theory has been well recognized[51]. Experimentally, Akazaki (1956)[50] observed that when typoid paratypoid vaccine was infused in intra-abdominal cavity, thereby the histiocytes collected, and 3 days after phagocytic activity of histiocyte which occurs in subcutaneous was seen without collection of neutrophilic leucocyte.

As inner factors, the following conditions are necessary for the occurrence of giant cells: remaining of foreign body for a long time to provide giant cells, for example, slightly soluble fragments of injured tissue, and the damage of the tissue[52]. In the present experiment the necessity of above factor was recognized. The elasticophage may occur at noticeable destroy of the elastic fibres[45][46]. Although an intense destruction of the elastica is seen, it is suggested that mesenchymal activation is necessary[23]. Gonzalez (1964)[53] showed, when aortic fibres were implanted in the homo-urinary bladder experimentally, neither granuloma reaction nor giant cell formation was observed, therefore, although there was an intense destruction and fragmentation of the elastica, no tissue reaction was seen. For the occurrence of elastogranuloma reaction with giant cell, the essential factor may be the destruction of elastica and some additional factors. Friedman and Byers (1964)[24] observed that endothelial cell on the thickened intima showed quickly phagocytic property.

Duß and Bostelman (1965)[54] showed that when animals obtained high sensibility of tissue reaction with the injection of the elastica and elastica plus Adjuvants, there was a clear difference in the occurrence of typical foreign body type giant cell between the injection with Adjuvants and without.

In the present experiment, although noticeable destruction of the vessel walls by elastase was obtained, therein no elastogranuloma reaction was seen. However, in the cases that the vaccine was injected, multinuclear giant cells

phgocytosing the fragments of disintegrated internal elastic lamina were recognized. In these cases the lumen was completely occluded with the thrombi and thickened intima in which proliferation of increased fibroblaste and histiocyte was seen. And from these experimental results, it was concluded that mesenchymal activation is an essential factor for giant cell formation.

The living period of giant cell is influenced by the nutrition, that is, in well fed tissue giant cell lives longer than in poor condition. And then, the necessity of nutrition for life of giant cells is well proved by Langhans's cell type giant cell in tuberculosis, because in the condition without vessels or poorly vascularized tissues, giant cell promptly disappeared. Giant cells have no particular tissue where they can live, then they disappears when it's phagocytic action is finished, sooner or later[49]. In the present experiment, in the cases of two and three weeks after the injury, elasticophagic giant cells were seen, but the cases of long term observation had no giant cell. The elastic fibres have a effect as fogeign body under a special condition[36)52)], so such a tissue reaction as increasing and wandering of fibroblaste and histiocyte may be provoked. Especially, the sharped and fractured elastica has an action as foreign body, and it provokes to elastogranuloma reaction[52)], but no giant cell reaction of newly elastica was seen[14)]. In the present experiment only at disintegrated elastica giant cell reaction was seen.

Etiology and pathogenesis of pulseless disease

In 1948 Shimizu and Sano demonstrated that pulseless disease is a clinicopathological entity. At present, it is called by many names: patho-morphologically, by the location of lesion and after investigator's name[55)]. Frövig and Löken (1951)[56)] summarized that this disease belongs to the category of aortic arch syndrome. According to Ross and McKusick (1953)[57)] it is designated as young female arteritis variety of aortic arch syndrome.

Signs and complains in pulseless disease varied with location of lesion, of which the most prominent signs are visual disturbances, hyperactive carotid sinus reflex, and ischemic symptoms in the upper limbs[1)4)]. Occasionally renovascular hypertension is seen. Gibbons and King (1957)[59)] said that this disease should be considered when cataracts were encountered in young people and when arterial pressures and pulses in the upper extremities were absent.

According to Koszewski (1958)[60)] the course of lesion is segmental and propagates to periphery. However, Sano and Aiba (1965)[61)] pointed out that thrombosis began in very characteristic sites, namely in a portion of both subclavian arteries distal to the vertebral ramification and in the distal part of both common carotid arteries just proximal to the bifurcation, and the segmental obstructions which could be seen in arteriosclerosis were not observed.

Although involvement of the common carotid, subclavian or innominate arteries are most frequently demonstrated, because the disease may occur

with equal intensity in the caudal stem branches, that is, the coeliac, superior mesenteric and renal arteries, the conception for the disease is enlarged, and pulseless disease and atypical coarctation are included in "Aortitis syndrome"[57)62)].

On the origin of the lesion, Shimizu and Sano (1948)[1)] offered the aorta origin theory, which tuberculous lesions in the hilus by way of the vasa vasorum that occur from aortic stem affect the adventitia of the carotid artery. Cook and Cloake (1946)[15)] took the vasa vasorum origin theory, and said that pathologic changes were results of secondary ischemia by the occlusion of the vasa vasorum. From the clinical and histological features Boström and Hassler (1965)[17)] proposed that the disease may start in the large arteries in the neck and in the descending aorta and upper abdominal aorta, and then progress to involve the whole thoracic aorta.

In the present case in which the surgical treatment was taken in the aortic arch, the aortic wall showed cell infiltration around the vasa vasorum, marked intimal thickening and destruction of the elastic fibres, so it is considered that the lesions primarily began in the aorta.

In the active stage, the lesion begins in the vasa vasorum of the adventitia, namely it is a neutrophilic periarteritis with secondary thrombi. In the specimens which were taken between two months and two years from the onset, those specific histological features were cell infiltration around the vasa vasorum of the adventitia in active stage, and remarkable fibrosis of the adventitia in advance stage. The vasa vasorum appear to be a primary sites of involvement of pulseless disease.

In abdominal aortas of dogs, intimal thickening and medial destruction were experimentally produced by the occlusion of the vasa vasorum[63)].

Ikeda (1966)[76)] reported that circulating antibodies against the outer layer of the aorta and pulmonary were demonstrated in Boyden's hemoagglutination reaction.

According to Gillman (1964)[67)], the mammary artery grows rapidly during lactation and then involutes, even more rapidly, with the cessation of lactation. Because the vasa vasorum in the brachiocephalic, innominate and subclavian arteries stem from the mammary artery, the dysfunction of the mammary artery may play a role in the pathological alteration of the major branches from aortic arch, by way of the vasa vasorum.

In advanced lesion, the scaring formation, intimal thickening and occasionally, aneurysm formation were seen[58)68)]. Because destruction of the elastica in the media results in fragility of the aortic wall, aneurysm in the right common carotid artery was revealed in arteriograph, and was recognized at operation.

Kalmonsohn and Kalmonsohn (1957)[64)] pointed out that no fibrinoid degeneration in pulseless disease, therefore, it is non-specific angitis which differs

from necrotizing angitis[65]. However, Ooneda (1965)[66] suggested that fibrinoid degeneration may occur because of hyperpermeability of the vessel wall, and in autopsy of a patients with pulseless disease, the presence of fibrinoid degeneration was recognized. In case 21, fibrinoid degeneration in the intimal thickening of the ascending aorta was observed (Fig. 5). In the present experiment when remarkable destruction of the internal elastica lamina by elastase infusion was seem, fibrinoid degeneration was observed.

Cases 10 and 20 correspond to diffuse productive inflammation type, and bear the similarity to Buerger's disease. However, the selective involvement of the aortic arch opposes such a conclusion that the disease is identical with thromboangitis obliterans or periarteritis nodosa.

Histo-pathologically, both pulseless disease and giant cell arteritis have the same features[46][48][64]. At least, the cases reported by Horton (1934)[44] were related with pulseless disease histologically, in spite of the fact that sex and age factors were different from those seen in pulseless disease[68].

Because two of four cases were reported by Gilmour (1941)[45] as giant cell arteritis which had involvement in the common carotid, subclavian and innominate artery and the cases reported by Cook (1946)[15] had lesions in the aorta, radial and subclavian arteries, and all the cases must be included in the category of young female arteritis by McKusick (1953)[57].

Tokoro (1962)[48], Austen (1965)[68], said that pulseless disease is identical with giant cell arteritis. Basu (1961)[69] said that the presence of giant cell could be regarded as indication of a primary affection of the connective tissue.

On the other hand, although a relationship between these diseases cannot be denied, it would be wiser for the present time to consider them as separate entities clinically.

In giant cell arteritis, Kimmelstiel and Gilmour (1957)[47] said that the intimal thickening, thrombus formation and inflammation of the vasa vasorum are secondary changes, and the original lesion is a peculiar destruction of the elastic fibres accompanied by giant cell reaction.

The lesion of the elastic fibres seems to play an important role on the pathogenesis of pulseless disease.

On the contrary to these elastica origin theory, Kunz (1963)[14] pointed out that degeneration and regeneration of the elastica, and changes of AMP are not specific in this disease. Also, Hamperl (1953)[52] said that if the elastic fibres are in the vicinity of destroyed tissues and are included in the granuloma, elasticophagic giant cells may be formed. Gonzalez (1964)[53] supported that for the occurrence of giant cell mesenchymal activation is an essential factor. In the present experiment, primary elasticopathy accompanied by granulomatous reaction with elasticophagic giant cells exists, and the following conditions are necessary for the occurrence of elasticophagic giant cells: a specific destruction of the elastic fibres to provoke giant cells for example,

hard and slightly soluble fragments of the internal elastic lamina, and a mesenchymal activation brought about by some factors.

Clinico-pathologically, there have been many hypothesis and suppositions for the etiology of this disease; tuberculosis[1], rheumatic disease[70], streptococcal infection[71], congenital malformation[72] and autoimmune mechanism[73].

From the histologic features of pulseless disease and the fact that seven cases had tuberculosis in histories, in the present cases, tuberculosis or allergy following tuberculous infection seems to be an important etiologic factor. Infection has been considered to play an important role on the process[62,70], however, no organism has not yet been discovered[1,71].

On the contrary to inflammation theory, many investigators[48,58] have taken up congenital malformation theory which was first proved angiographically by Elliot (1939)[72].

Dalith (1963)[75] showed that developmental structural deficiency of the arterial wall of the brachial artery derivatives, at six points of previous connection of embryonic vascular structures, suggested the aortic arch and the brachiocephalic arteries. However, Kalmonsohn and Kalmonsohn (1957)[64], Gibbons and King (1957)[59], and Judge (1962)[55] denied the congenital malformation.

By Gillman (1964)[67], such a possibility was presented that the striking differences in the structure of susceptible parts of the aorta may be a function not only of hemodynamic factors but also of the peculiarities in metabolic activity and turnover rates of constituents of the walls of susceptible arteries. And deranged remodelling of the aorta was indeed responsible for its susceptibility injuries.

The investigators who adopt the collagen disease as etilogy, pulseless disease is urged to clarify whether it's lesion is a local feature of the systemic disease or not[61].

From specific facts that pulseless dieseas is seen mostly in women, the abnormality of sexual hormone is pointed out[55]. Although the peripheral vascular diseases are seen mostly in men, pulseless disease in women[61], erythema nodosum in women, and lups erythematosis disseminates in young women[70].

Estrogen has anti-atherosclerotic action to the vessel walls[41,42]. These facts have been described on sex specificity in vascular diseases. Goover (1965)[77] reported that estradiol induces intimal thickening in young male rat, while testosteron has the relationship with increase of hematocrit and formation of clot. Thereby, he pointed out the relationship between vascular disease and sexual hormon.

In the present experiment (C), increased elastic stainable material in the thickened intima was seen, and the thickened intima and the media reacted as metachromatic, in which metachromatic material was mainly hyaluronic acid, and such changes were weaker in old female rabbits, and specific action

of estrogen to vessel walls was observed. However, Goover's result (1965) was not confirmed.

In recent years, Stein (1965)[54] indicated that anti-elastin antibodies are present in human sera, and the absorption of these antibodies from the circulation blood to the elastic fibres in the vessel wall or elsewhere has also to be considered. In the tissue in which hyperimmune reaction was previously produced by vascular elastin, the vascular destruction will occur without great difficulty by local stress as highly velocity or eddy current[73]. Because the elastic fibres have properties as protein, their antigen action may yield hypersensibility of the subject to the organism[54].

From above findings and present experimental results, the elasticopathy related with anti-elastin autoimmune mechanism may be main etiologic features.

The relationship between elasticopathy and auto-immune mechanism or allergy following infection should be investigated.

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EXPLANATION OF FIGURES

FIG. 1. Cross section of right common carotid artery (case 18). Showing marked destruction of muscle fibres in intimal thickening, media and remarkable fibrosis in the adventitia with occlusion of the vasa vasorum.
Hematoxylin and eosin. ×100.

FIG. 2. The same section as in Fig. 1. Destruction and disappearence of muscle fibres in the media are marked. Note newly formed elastic fibres in the thickened intima.
Weigert's elastic fibre stain. ×100.

FIG. 3. Longitudinal section of ascending aorta (case 21). Showing remarkable fibrous thickening in the intima, irregularity in the arrangement of the muscle fibres in the media with cellular infiltration, and occulsion of the vasa vasorum and fibrosis in the adventitia.
Hematoxylin and eosin. ×100.

FIG. 4. The same section as in Fig. 3. Showing intense destruction and fragmentation of the elastic fibres in the media, and newly formed elastic fibres in the thickened intima.
Weigert's elastic fibre stain. ×100.

FIG. 5. The same film as in Figs. 3 and 4. Fibrinoid degeneration is seen at the luminal portion in the thickened intima.
Mallory's phosphotungstenic hematoxylin. ×100.

FIG. 6. Cross section of right subclavian artery (case 7). Tuberculoid granuloma formation in the media showing disappearrance of muscle fibres and infiltration of lymphocytes, plasma cells, epitheloid cells and giant cells.
Hematoxylin and eosin. ×400.

FIG. 7. The same section as in Fig. 6. Degenerated elastic fibres are surrounded by epitheloid cells, elasticophagic giant cells and round cells.
Weigert's elastic fibre stain. ×400.

FIG. 8. Cross section of right common carotid artery (case 13). Showing granulomatous inflammation in which numerous elasticophagic giant cells in the media.
Hematoxylin and eosin. ×100.

FIG. 9. The same section as in Fig. 8. Showing mulitnucleated giant cells phagocytosing the fragments of disintegrated elastic fibres.
Weigert's elastic fibre stain. ×100.

FIG. 10. Cross section of right axillar artery (case 10). Note the diffuse productive inflammation in all layers. The lumen is occluded by organized thrombus.
Hematoxylin and eosin. ×100.

FIG. 11. The same section as in Fig. 10. Showing elasticophagic giant cells in assosiation with distruction of elastic fibres in the media.
Hematoxylin and eosin. ×400.

FIG. 12. The film of common carotid artery of the rabbit which was incubated by 0.1% elastase suspension for 20 min's, destruction of elastic fibres is observed at luminal portion in the media.
Weigert's elastic fibre stain. ×400.

FIG. 13. The same experiment as presented in Fig. 12. 30 min's after the incubation, the destruction of the elastic fibres in the entire media is recognized.
Weigert's elastic fibre stain. ×400.

FIG. 14. The film of the vessel which was incubated by 0.1% trypsin solution for 60 min's, elastic fibres remain intact.

FIG. 15. Cross section of common carotid artery of the rabbit, fifteen days after submitting the vessel to the action of 0.3% elastase suspension for 3 hours. The lumen is completely occluded with organized thrombus and intimal thickening. Note elastolysis and occurrence of elasticophagocyting giant cells.
Weiget's elastic fibre stain. ×400.

FIG. 16. Cross section of common carotid artery of the rabbit, twenty-one days after the same experiment as pressnted in Fig. 15. Note an intense destroy of elastic fibres and elasticophagic giant cells in the media.
Weigert's elastic fibre stain. ×400.

FIG. 17. Cross section of common carotid artery of the rabbit, thirteen days after the injury. There is marked and unequal thickening of the intima in which elastosis and elastoidosis are seen. The lumen is still patent.
Weigert's elastic fibre stain. ×100.

Fig. 18. Cross section of common carotid artery of the rabbit, three months after the injury. Showing remarkable thickening of the intima with elastoidosis and newly fomed elastic fibres. Note that a degraded internal elastic lamina remains as a fragment, but neither regeneration nor reconstruction of destroyed elastic fibre are seen in the media.
Weigert's elastic fibre stain. ×100.

FIG. 19. Cross section of common carotid artery of the rabbit, ten days after the periadventitial injection of 0.3% elastase suspension. Note the disarrangement and the degeneration of muscle cells in the media, and thickening of the intima. Transformation of the mucsle cell to brilliant cell is seen.
Hematoxylin and eosin. ×400.

FIG. 20. Cross section of common carotid artery of the rabbit, fifteen days after the injury by the same experiment as in Fig. 22. The lumen is still patent and the intima is thickened.

Hematoxylin and eosin. ×100.

FIG. 21. The same section as in Fig. 23. Showing intimal thickening with the elastosis, and the destroy of elastic fibres in the media.
Weigert's elastic fibre stain. ×100.

FIG. 22. Cross section of common carotid artery, seven days after submitting the vessel to the action of 0.3% trypsin solution for 3 hours. Showing the lumen is still patent, elastic fibres remain intact and muscle fibres disappear.
Weigert's elastic fibre stain. ×100.

FIG. 23. Cross section of common carotid artery of the rabbit, for contral three weeks after submitting the vessel to the action of 0.2-MOl borate buffer was infused. Showing no degeneration in the arterial wall.
Weigert's elastic fibre stain. ×100.

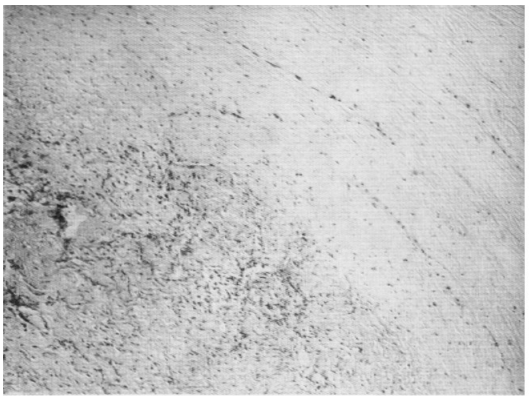


FIG. 1

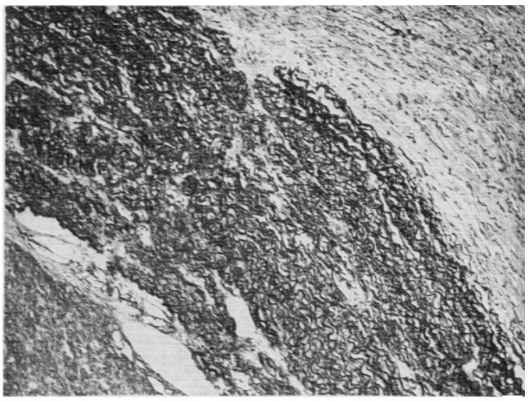


FIG. 2

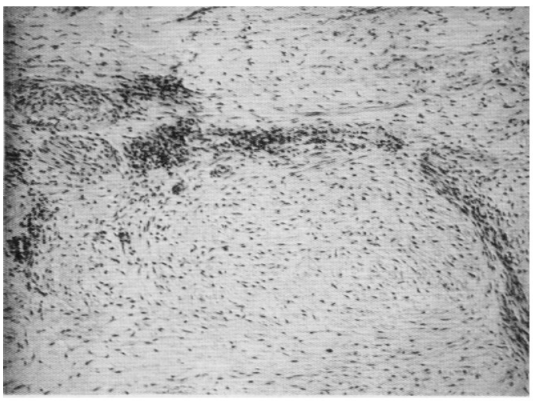


FIG. 3

FIG. 4

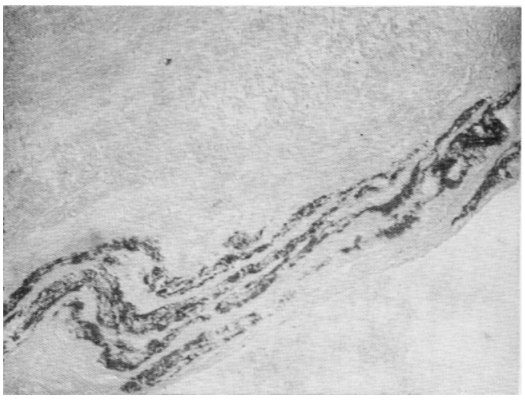


FIG. 5

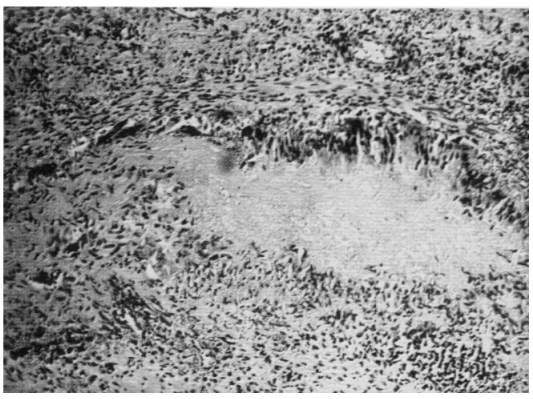
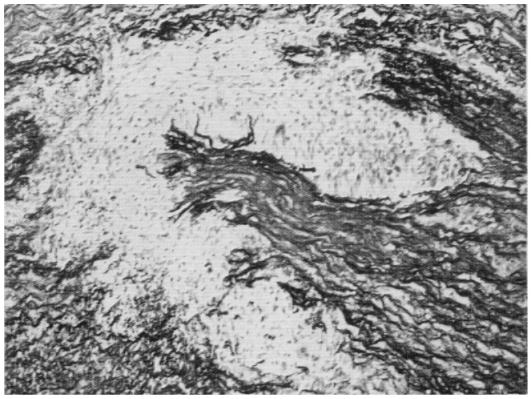


FIG. 6



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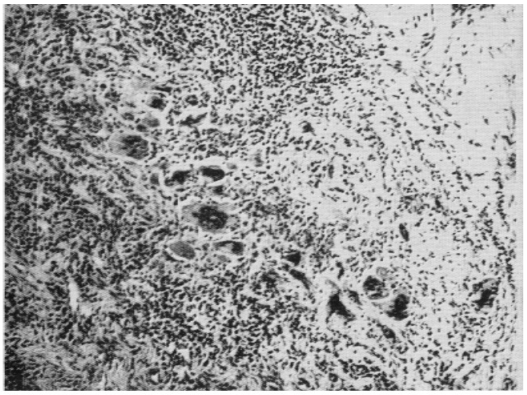

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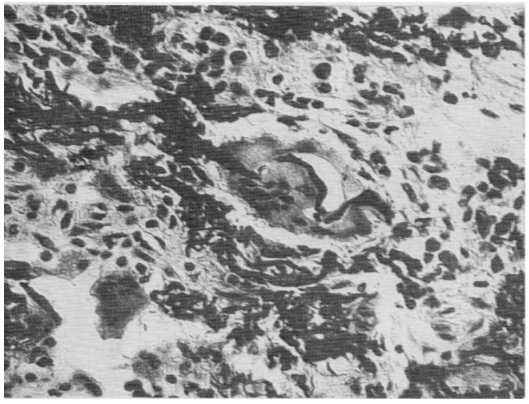

FIG. 9

FIG. 10

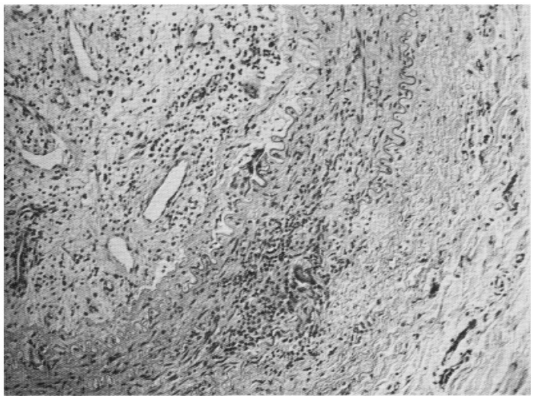

FIG. 11

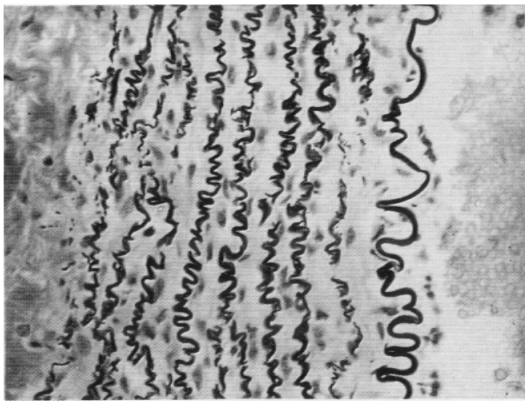

FIG. 12

FIG. 13

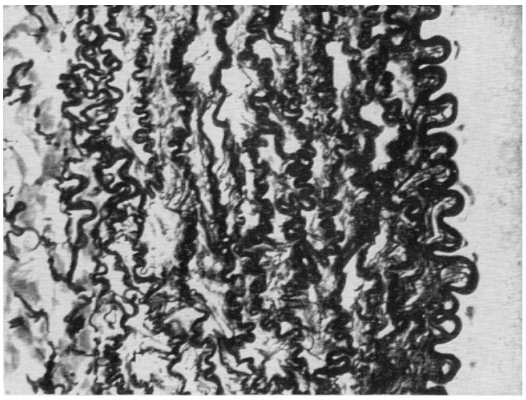

FIG. 14

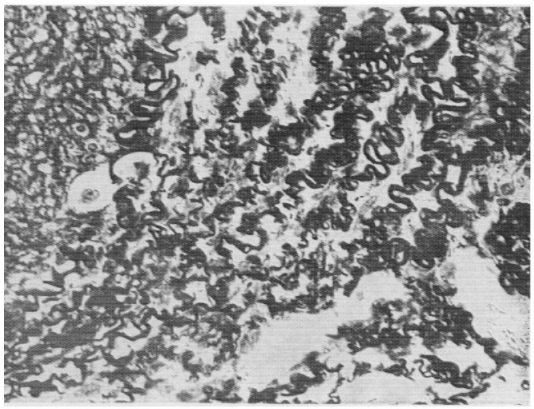

FIG. 15

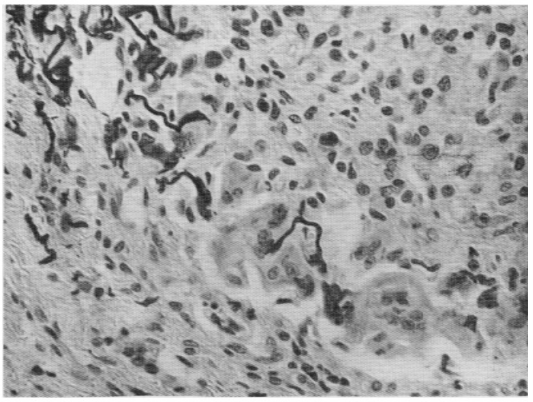

FIG. 16

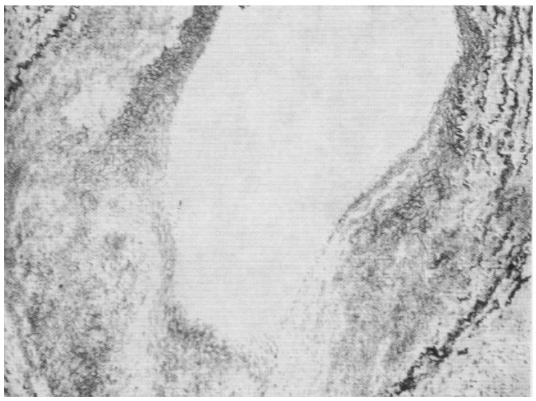

FIG. 17

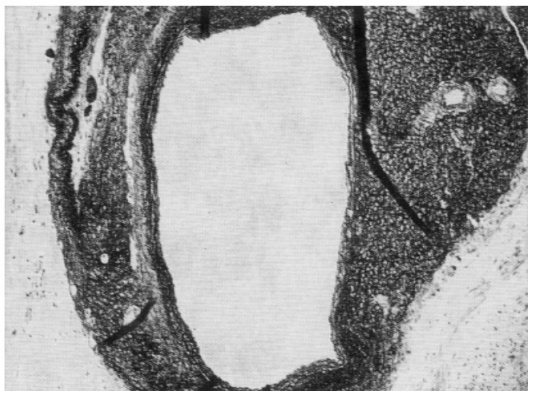

FIG. 18

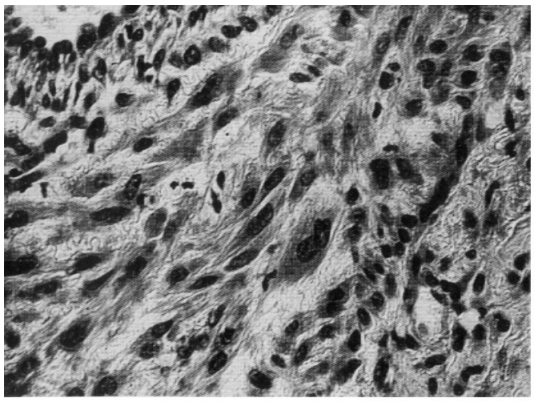

FIG. 19

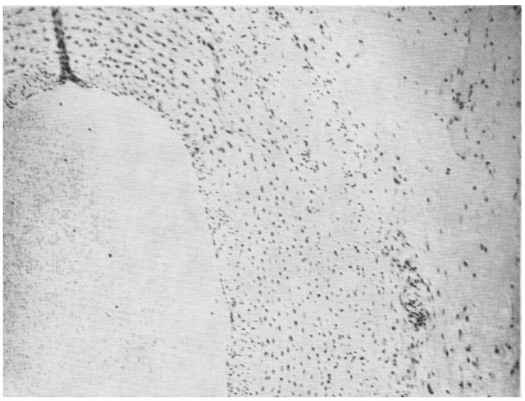

FIG. 20

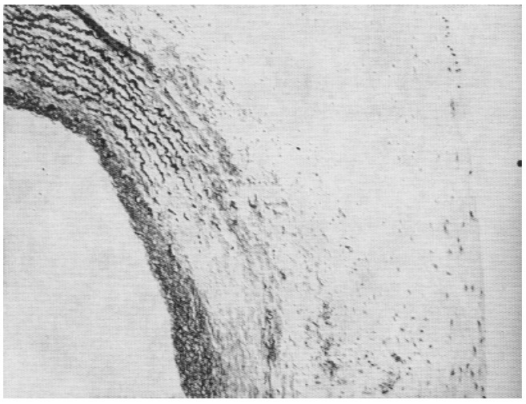

FIG. 21

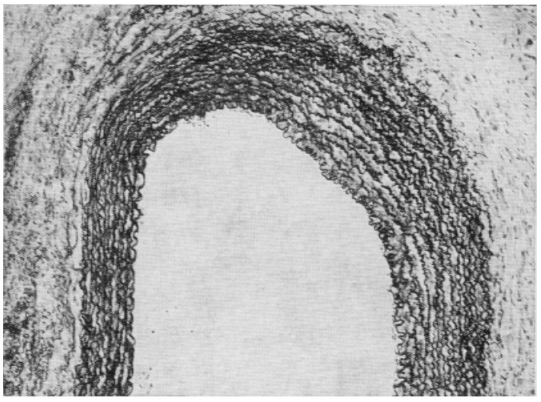

FIG. 22

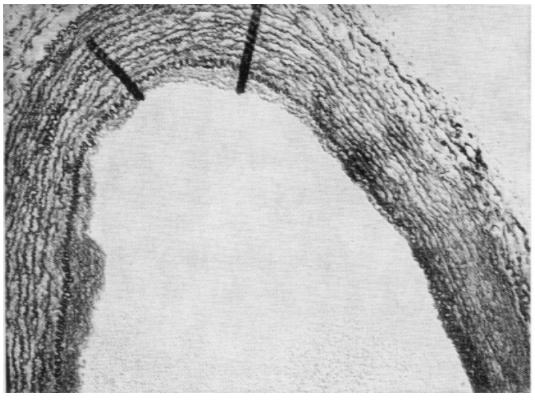

FIG. 23