ATYPICAL AMYLOID DISEASE, WITH OBSERVATIONS ON A NEW SILVER STAIN FOR AMYLOID *

LESTER S. KING, M.D.

(From the Laboratory of the Illinois Masonic Hospital, and the Department of Pathology, University of Illinois College of Medicine, Chicago, Ill.)

There has been no general agreement on the classification of amyloid disease. Not only is there a bewildering array of names, such as primary, secondary, atypical, local, systemic, tumor-forming, and paramyloidosis, but there is no uniformity in regard to the meaning or scope of these terms. One of the most interesting manifestations of the disease has been described as primary amyloidosis, a term especially popular in the American literature, indicative of no known cause or pre-existing disease which might be held responsible for the amyloid deposition. The two most complete reviews of the subject are the papers of Koletsky and Stecher [1] and, more recently, of Lindsay.[2] Koletsky and Stecher listed 22 cases, as of 1939, while Lindsay in 1946 found 45. Several other reports are mentioned by Lindsay but not considered acceptable by him. In the absence of satisfactory criteria, it may be stated that approximately 50 cases are described in the literature.

The purpose of the present communication is threefold: To present 6 cases of unusual amyloid disease, 5 of which appear similar to the rare primary form; to describe a new silver stain for amyloid; and to analyze the confusing nomenclature and terminology, and suggest a new classification.

REPORT OF CASES

(Case 1 is from the Fairfield State Hospital, Newtown, Connecticut, obtained through the courtesy of Dr. W. F. Green, Superintendent. The other 5 cases are from the Illinois Masonic Hospital, Chicago, Illinois.)

Case 1

The patient was a white male, 88 years old. He was a pauper, brought to the Fairfield State Hospital showing marked mental deterioration in all spheres. The heart sounds were of poor quality, and severe peripheral vascular sclerosis was present. The blood pressure was 140/70 mm. Hg. Moderate tremor of hands and feet was noted. Physical examination was otherwise negative. The psychiatric diagnosis was psychosis with cerebral arteriosclerosis. Laboratory data showed negative serologic findings, a red blood cell count of 4,400,000; 81 per cent hemoglobin, and a white blood cell count from 8,600 to 10,400, with a normal differential. The urine revealed occasional casts and a 1 plus albumin, otherwise negative. The nonprotein nitrogen of the blood was 28 mg. per cent. The patient succumbed to bronchopneumonia 3½ months after admission.

* Received for publication, October 21, 1947.
Presented at the Forty-Fifth Annual Meeting of The American Association of Pathologists and Bacteriologists, Philadelphia, March 12, 1948.

The final pathologic diagnoses were: Senility; senile plaques in brain; fibrosis of spleen, pancreas, and testes; brown atrophy of liver; amyloid in heart; bronchopneumonia; fibrinous pleuritis; acute passive congestion of liver; fibrous perisplenitis; accessory spleen.

The heart weighed 350 gm. Slight calcification was present at the bases of the aortic cusps, but the free margins were delicate. No gross scarring was observable. The coronary arteries, although tortuous, revealed only mild atheromatous deposits. The aorta was the seat of only slight atheroma. Arteriosclerosis was not considered as a significant part of the disease picture. The microscopic examination of the heart is discussed later. No amyloid was noted in any other organ. The other diagnoses need no comment.

Case 2

An 83-year-old man was admitted in a somewhat disoriented condition with a history of urinary difficulty, consisting of nocturia, difficulty in starting the stream, and dribbling, of uncertain duration. On physical examination the significant findings were some increased resonance in both lungs, moderate kyphosis, and scattered râles in the right lung. The heart was moderately enlarged to percussion, and a soft systolic murmur was present at the apex. The prostate was enlarged, symmetric, firm, nontender, and smooth. The blood pressure was 140/ 80 mm. Hg. Slight pitting edema of the lower extremities was present. Urine showed 3 plus albumin, no sugar, and many pus cells. The red blood count was 3,620,000, with but 38 per cent hemoglobin; the white blood cell count was 9,720, with a normal differential. Nonprotein nitrogen of the blood was 47 mg., creatinine 1.6 mg. per cent. The heart action was irregular. The electrocardiogram showed auricular fibrillation and diffuse myocardial damage. There was roentgenologic evidence of Paget's disease, involving the skull, spine, femora, and pelvis. The alkaline phosphatase value was 9.8 Bodansky units and the acid phosphatase, 1.6 units. The phosphorus was 3.8, and the calcium 7.5 mg. per cent. The patient was given numerous transfusions. Following these the red blood cell count was 4,000,000; the color index, 0.8. At operation, an extensive carcinoma of the bladder, of papillary type, was excised, and the prostate removed. Pathologic examination showed benign hyperplasia of the prostate, and papillary carcinoma of the bladder, transitional cell type. The patient did not do well after operation. His nonprotein nitrogen gradually rose to 172 mg. per cent with creatinine of 5, and he expired 14 days after operation.

The pathologic diagnoses following autopsy were: Recent operation (prostatectomy and removal of carcinoma of bladder); necrotizing cystitis; chronic pyelonephritis; generalized arteriosclerosis; calcific aortic sclerosis (Mönckeberg type), mild; myocardial hypertrophy and fibrosis; amyloid in heart; chronic passive congestion of liver; congestion and edema of lungs; Paget's disease of bone.

The heart weighed 470 gm. and was dilated in all chambers. Considerable calcification was present in the sinuses of Valsalva, and in the bases of the aortic cusps and mitral ring. The free margins of the valves were delicate. Extensive calcification and arteriosclerosis were

present in the coronary vessels, as well as in the aorta and major arteries. Microscopic study of the heart is discussed later. The left kidney weighed 70, the right, 180 gm. Both were coarsely granular, with obscuration of the corticomedullary junction, with severe fibrosis, and interstitial infiltrations of lymphocytes and plasma cells. Some acute inflammatory changes were present in collecting tubules and pelvic mucosa, but these were relatively slight. The skeletal changes were typical of Paget's disease but relatively inactive. No amyloid was noted except in the heart.

Case 3

The clinical record on case 3 is inadequate. The patient, an irrational 87-year-old woman, died 30 hours after admission. On entry her abdomen was markedly distended, the legs were edematous. Temperature on admission was 97° F. The clinical impression was arteriosclerotic heart disease. The red blood cell count was 4,900,000; hemoglobin, 14.1 gm.; white blood cell count 23,250, with 91 per cent polymorphonuclear leukocytes. Serologic findings were negative. Nonprotein nitrogen of the blood was 57; urea nitrogen, 32 mg. per cent. Paracentesis was performed and approximately 500 cc. of turbid fluid were removed. The patient remained irrational. Her temperature never rose above 97° F. and was recorded at 96° F. shortly before death.

The final pathologic diagnoses were: Generalized arteriosclerosis; calcific aortic sclerosis (Mönckeberg type); acquired bicuspid aortic valve; cardiac hypertrophy and dilatation; myocardial fibrosis; amyloid in heart; chronic passive congestion of lungs; amyloid in lungs (slight); pleural effusions; cardiac cirrhosis of liver; edema of ankles; infarct of spleen; generalized peritonitis, cause undetermined; slight bronchopneumonia; transitional cell carcinoma of vagina.

The two major disease sequences could not be brought into any definite relationship. The peritonitis was generalized and severe, but no cause therefor could be ascertained. The gastro-intestinal and genito-urinary tracts were anatomically intact. No primary focus of infection could be identified. The bronchopneumonic patches in the lungs did not appear antecedent or causal to the peritonitis. The splenic infarct was bland, and not septic. It was suggested that perhaps, in the patient's enfeebled condition, peritoneal infection might have occurred from the lumen of the bowel through an anatomically intact wall.

The circulatory system proved of great interest. The heart weighed 630 gm. The aortic valve was the seat of severe calcific sclerosis, most pronounced at the bases of the cusps. A bicuspid valve was produced. The free margins of the leaflets were pliable, although slightly thickened. Calcareous deposits at the bases of the mitral cusps also were present. Fine grayish streaking was visible in the myocardium. Microscopic findings are discussed later. The findings in liver and lungs were

indicative of long-continued cardiac insufficiency, with prominent chronic passive congestion, reaching the intensity of cardiac cirrhosis in the liver. In the lung small amounts of amyloid were noted in the thickened alveolar walls, but none was seen in any other organ. A small, recent, bland infarct of the spleen was considered to be arteriosclerotic in origin. The kidneys weighed about 120 gm. each and appeared excellently preserved.

An incidental finding was a small nodule, about 1.5 by 1 cm., situated in the posterior vaginal wall at the posterior fornix, which on section proved to be a papillary carcinoma of transitional cell type. It was superficial and did not invade the vaginal wall.

Case 4

The patient was a male, 93 years old, who had had two hospital admissions. On the first his complaints were dyspnea on exertion for about 3 weeks, progressive weakness for 3 months, and black stools for 2 weeks. The significant physical findings were: Enlargement of the heart to the left, with extrasystoles and a harsh apical systolic murmur. The blood pressure was 150/80 mm. Hg; red blood cell count, 2,550,000, with 43 per cent hemoglobin; white blood cell count, normal. Nonprotein nitrogen of the blood was 47 mg. per cent, but creatinine and sugar were normal. The electrocardiogram was interpreted as showing severe myocardial damage. Stool examinations showed occult blood. After repeated blood transfusions a gastro-intestinal x-ray series was made, showing an apparent gastric ulcer on the lesser curvature. The patient's condition improved after appropriate therapy, and he was discharged 17 days later. The clinical impression was: Bleeding peptic ulcer of stomach; organic heart disease with mitral insufficiency and mild congestive failure.

The patient was re-admitted 7 weeks later because of pain, redness, and swelling of the left leg, of 3 weeks' duration. He had been relatively well on digitalis in the interim. Examination showed thrombophlebitis of the left leg; slight auricular fibrillation; blood pressure, 150/76 mm. Hg; red blood cell count, 4,700,000, with 70 per cent hemoglobin; white blood cell count, 16,500, with 85 per cent polymorphonuclear cells. Nonprotein nitrogen of the blood was 24 mg. per cent. The patient was treated with dicumarol, the prothrombin time being regulated at 35 to 40 per cent of normal. The thrombophlebitis showed marked improvement. However, 13 days after the final admission, the patient died rather suddenly.

The pathologic diagnoses after autopsy were: Generalized arteriosclerosis; myocardial fibrosis; myocardial hypertrophy; amyloid in heart; dilatation of heart; chronic passive congestion of lungs; amyloid in lungs, slight; thrombophlebitis, left femoral vein; infarct of spleen; petechial hemorrhages of intestinal serosa and pelvis of kidney. No residuum of a peptic ulcer was encountered. No pulmonary embolus was present.

The heart weighed 420 gm. Considerable epicardial fat was noted. There was moderate dilatation of the chambers and some hypertrophy of the musculature. The valves were delicate, except for slight nodular thickening of the free edges of the mitral cusps, but the chordae ten-

dineae were delicate and inserted normally. The coronary arteries, patent throughout, were tortuous, but showed no calcification and only slight atheroma. Notation was made that they appeared extraordinarily well preserved for a man of this age. Dense fibrosis was noted at the tips of the papillary muscles, and a few minute, translucent, grayish dots and fine streaks were observed in the myocardium, especially of the septum. The aorta showed mild sclerotic changes. The microscopic findings in the heart are described later. The left femoral vein was occluded by a thrombus, and chronic inflammatory infiltrations were present in the thickened wall. The splenic infarct was small, measuring only 1.5 cm. across, and was recent. Moderate chronic passive congestion involved the lungs. Small traces of amyloid were seen in the thickened alveolar walls, but not in any other organ. The petechial hemorrhages of the intestinal serosa and renal pelvis were apparently agonal.

Case 5

The patient was a woman, 88 years old, who was admitted following a fall at home, in which she fractured her right hip. The fracture was inter-trochanteric, and comminuted. Physical examination otherwise was not remarkable, except for an apical systolic murmur. With the extremity in traction, the patient was placed on sulfonamide therapy and her general progress was satisfactory. The terminal episode, 3 months after admission, was ushered in by a sudden hemorrhage from the rectum. In spite of supportive therapy, she died within a few hours.

The pathologic diagnoses were: Generalized arteriosclerosis; occlusion of inferior mesenteric artery; infarction of distal transverse colon, sigmoid, and rectum; nephrosclerosis, arteriosclerotic type; old healed infarct of lung; amyloid in heart and lung; fibrous perisplenitis; portal cirrhosis of liver, mild; bullous emphysema, moderate; atelectasis, right middle and lower lobes; lymphocytic infiltration of adrenals; parovarian cysts, bilateral; old fracture of the right hip.

The heart weighed 230 gm. Fine grayish streaks were noted in the myocardium. The aortic valve showed slight calcareous deposits at the base of the cusps. The coronary vessels were severely sclerotic, but the lumina were only moderately narrowed. The microscopic findings are considered below. The aorta revealed extensive ulceration and calcification, and the ostium of the inferior mesenteric artery was occluded. The other major arteries were affected by severe atherosclerosis and calcification. The infarction of the distal colon, sigmoid, and rectum was characteristic. The lungs, with senile emphysema and some bullous formation, weighed only 190 and 150 gm. respectively. Some atelectasis also was present. One minute area of fibrosis, with fibrotic occlusion of a nearby artery, was interpreted as an old healed infarct. Traces of amyloid were present within the media of the

fibrotic blood vessels in this area. None was seen elsewhere, neither in other parts of the lung nor in other organs. The liver weighed 1000 gm. The surface was slightly granular, and microscopic examination showed a distortion of architecture by a mild excess of fibrous tissue, radiating irregularly from the portal spaces. The nephrosclerosis, with kidneys weighing 105 and 115 gm., was characteristically arteriosclerotic in type. In the pancreas small areas of fibrosis affected the interstitial connective tissue, but the islands were intact.

MICROSCOPIC STUDY OF HEARTS

These 5 cases appear to form a single group and may be considered together. The ages of the patients ranged from 83 to 93 years. The causes of death varied greatly. The weights of the hearts ranged from 230 to 630 gm. The heart weighing 630 gm. showed severe Mönckeberg's sclerosis of the aortic valve, with an acquired bicuspid valve, and the hypertrophy seemed a direct result of this. Three of the hearts with weights of 420, 470, and 630 gm. showed considerable interstitial fibrosis but the other 2 (230 and 350 gm.) revealed no significant fibrosis within the myocardium. Arteriosclerotic changes, generalized, were prominent in all but case 1, but less so in case 4 than in cases 2, 3, and 5.

The microscopic examination of the hearts revealed fundamental similarity. In all there were small patchy masses of hyaline material, situated in the interstitial tissue, and surrounding muscle fibers singly and in groups. This hyaline material in all specimens reacted positively with Congo red and with methyl violet. The latter stain gave intense metachromatic reactions, while the Congo red reaction was less strong. The hyaline substance also stained positively with ammoniacal silver, according to the method given below. The general pattern of the amyloid, in all cases, is illustrated in Figures 1 and 2. The hyaline material (Fig. 1) at first glance suggests fibrosis. In foci where the involvement is intense, the muscle fibers have disappeared or are markedly shrunken and atrophic, as if choked by the surrounding collars of amyloid. Careful examination, however, shows absence of fibrillar texture characteristic of collagen, while the deposits are hyaline. Differential staining reactions are, of course, conclusive.

Where variable amounts of interstitial fibrosis were present, amyloid was observed not only in immediate contact with muscle fibers but also as occasional small plaques and masses of hyaline material within the fibrosed zones. These deposits of amyloid within areas of fibrosis were readily recognizable in preparations stained with hematoxylin and eosin, and were differentially shown by specific stains. Methyl violet

was most useful in this regard. The heart of case 3 revealed the most marked interstitial fibrosis in this series.

It must be emphasized, however, that in none of these cases was the total amount of amyloid very great, and certainly not comparable to the massive deposits reported occasionally in the literature. The hypertrophy in the largest heart in this series, 630 gm., was explained by the aortic valvular disease. In all 5 cases the amyloid appeared to be an incidental finding. There was not sufficient evidence to attribute death to the deposition of amyloid.

In cases 3, 4, and 5 small amounts of amyloid were noted in the lungs. In cases 3 and 4, chronic passive congestion of the lungs was present, and the small amyloid masses were situated in the thickened alveolar septa. In case 5 the hyaline substance was seen within arterial walls in a zone of old infarction. In none of the cases was amyloid observed in any other organ.

AMYLOIDOSIS WITH PYELONEPHRITIS

A sixth case of atypical amyloid disease appears to be in a totally different category, but may be reported at this time.

Case 6

The patient, 70 years of age, speaking practically no English, was admitted in a critically ill condition, with nausea, vomiting, and severe abdominal pain of about 1 week's duration. The clinical impression was acute intestinal obstruction. Laboratory data showed a white blood cell count of 22,700, with 95 per cent polymorphonuclear leukocytes, and 4 plus albumin in the urine, with many pus cells. He had had previous surgical treatment, as evidenced by old abdominal scars. Although his physical condition was poor, immediate laparotomy seemed indicated. Severe adhesions were encountered and freed, releasing kinked and adherent loops of small bowel. An enormously distended gallbladder was noted. This was drained, and the abdomen closed. In spite of supportive postoperative therapy, the patient died 20 hours after admission.

It was found, subsequently, that the patient had had a number of hospital admissions in various hospitals, over a period of at least 12 years. He had had a resection of the cecum and ascending colon, with ileocolostomy, for carcinoma of the colon, verified pathologically. Apparently, he had a partial resection of the stomach, with anterior gastrojejunostomy at another hospital, but this record has not been verified. In the 12-year period there were numerous hospital admissions for vomiting, pain in the upper abdomen, dizziness, abdominal discomfort, anorexia, fatigue, chest pain, and vague symptoms. There was some evidence that over the past 12 years numerous urine examinations showed albumin and white blood cells, but no particular significance had been attached to them.

The pathologic diagnoses following autopsy were: Nephrolithiasis, right; severe subacute and chronic pyelonephritis; chronic ureterocystitis; atypical amyloidosis, involving kidneys, and blood vessels of numerous organs; arteriosclerosis of coronary arteries; healed infarct of heart; chronic passive congestion of lungs; atelectasis; emphysema;

scars of old operations (partial colectomy with ileocolostomy for carcinoma of colon, gastrojejunostomy with partial gastric resection); recent cholecystostomy and separation of adhesions.

The right kidney, intact, weighed 250 gm. It contained a large staghorn calculus, tightly surrounded by a rim of renal tissue. The dilated calyces contained abundant, greenish, purulent material. The persisting renal tissue, of greenish red color, showed severe reduction of the usual markings and of definition between cortex and medulla, and presented a somewhat mottled appearance. The right ureter had a diameter of 1.3 cm., with a dense, firm wall and a minute, slit-like lumen. The left kidney, weighing 220 gm., was granular, with a soft, bulging cut surface showing dulling but not obscuration of the renal markings. No hydronephrosis or calculi were present on the left and the left ureter appeared normal. The bladder was thin-walled, but with severe congestion of the mucosa. The prostate revealed slight hyperplasia. The heart weighed 370 gm., and showed an old healed infarct of the posterior left ventricular wall, extending into the septum. The coronary arteries exhibited extensive sclerosis and narrowing, but total occlusion was not discovered. Microscopic examination showed severe scarring and cellular infiltrations of the right kidney, with only slight fibrosis and pyelonephritis of the left. The right ureter and bladder revealed prominent chronic inflammatory changes and fibrosis. In both kidneys the glomeruli were involved by massive deposits of amyloid, of the usual subendothelial type (Fig. 3). In addition, the capsules of Bowman frequently were thickened by fibrous tissue, and many glomeruli were sclerosed and fibrotic without amyloid infiltrations. No amyloid was seen in relation to the tubules or interstitial connective tissue, but many of the small and medium-sized blood vessels contained lumpy hyaline masses in their walls. These masses stained positively for amyloid (Fig. 4). In the other organs amyloid was found in the blood vessel walls (similar to Figs. 4 and 5), in the heart, liver, gallbladder, gastro-intestinal tract, adrenal capsule (Fig. 5), ureter, and bladder. None was observed in the parenchymatous tissues of these organs, but only in the walls of arteries and veins. None was seen in relation to capillaries or sinusoids. No amyloid could be identified in any part of the lung, spleen, or pancreas. In the heart the amyloid was observed only within the walls of the coronary vessels. None was observed surrounding the muscle fibers, as in cases 1 to 5.

In summary, this case, with very long-standing pyelonephritis and nephrolithiasis, showed amyloidosis limited to the renal glomeruli and to arteries and veins in many different organs.

Classification of Amyloid Disease

Without attempting an exhaustive review of the literature, certain facts stand out clearly. The most common manifestation of amyloid disease occurs in the course of tuberculosis. Perla and Gross,[3] in a study of 1500 autopsies, reported 100 cases representing about 25 per cent of all patients dying of tuberculosis. In this form of the disease, the amyloid is most commonly distributed in the kidneys, liver, spleen, and adrenals; less frequently in one, two, or three of the above organs, but not in all.[4] Huebschmann [5] reported that in 8 of 9 consecutive autopsies on tuberculous patients with amyloidosis, the amyloid was found in the heart also. In addition, the amyloid may be found in traces in other organs, such as parts of the gastro-intestinal tract, pancreas, or salivary glands.[6] Thus there is a certain pattern of distribution, which I wish to call *typical*, that is, predominantly in the parenchyma of kidneys, spleen, liver, and adrenals, less frequently and with less intensity in certain other sites. This typical form of distribution, called secondary in the literature, is seen in the course of tuberculosis, osteomyelitis, pyelonephritis, lung abscess, carcinoma of stomach,[2, 7] carcinoma of lungs, leukemia,[3] tabes,[3] Hodgkin's disease,[7, 8] multiple myeloma,[9] rheumatoid arthritis,[3, 4, 10] thermal burns,[11] and others.

The use of the term secondary for this type is entirely misleading. It implies that, somehow, the associated disease (tuberculosis, for example) is the cause of the amyloid. A philosophic discussion of causality is scarcely relevant to the present paper. One must distinguish, however, a proximal or immediate cause, which is both necessary and sufficient (for example, prolonged local anoxia as a cause of infarction); and a more remote link in the causal chain (for example, generalized arteriosclerosis as a "cause" of infarction) which is neither necessary nor sufficient to produce the given phenomenon, but which may initiate or induce the proximal cause.

In this sense it is obvious that the proximal cause of amyloidosis is not known, in spite of considerable experimental study and chemical analysis. However, the statistical correlation between tuberculosis and secondary amyloidosis is too high to be ignored. One must assume, therefore, that tuberculosis, while not the direct cause, is probably part of the causal chain, provided that certain other unknown factors also are present.

The rôle of chronic suppurative disease in the production of amyloidosis is probably similar to that of tuberculosis. However, the position of conditions such as arthritis or Hodgkin's disease is more

questionable. There simply is not sufficient evidence to implicate these diseases as part of the causal chain, although they cannot arbitrarily be excluded. Judgment must be suspended.

This necessity for suspending judgment is emphasized by the rare case of amyloidosis, with essentially typical distribution, in which no other disease is observed. In various published cases,[12-16] for example, no associated disease was present, but the distribution of amyloid was approximately comparable to that seen in the so-called secondary type. Since we are ignorant of the true cause of amyloidosis, to call one group secondary and the other primary seems illogical. One might suggest that the true proximal (but unknown) cause was the same in both groups. In the alleged secondary group an associated disease might or might not be a remote factor of the causal chain; in the so-called primary group the causal chain is unknown throughout. In some instances, *e.g.*, case 2 of Dillon and Evans,[15] an associated disease (bacterial endocarditis) was present, but the authors nevertheless considered the case primary. In our state of ignorance such nomenclature is utterly confusing.

There is a second major category of amyloid disease which differs from the preceding in the sites of deposit of the amyloid. This category includes most of the so-called primary or atypical cases. Lubarsch,[6] and others, have discussed this group. The important criteria are: (1) Deposition of amyloid in unusual sites (heart, lungs, striated and smooth muscle, skin); (2) sparing of the usual or typical sites (spleen, kidneys); (3) absence of demonstrable cause. Inconstancy of staining reactions may or may not be present. The literature of these cases has been most admirably reviewed by Lindsay,[2] and repetition is unnecessary.

Since the cause of amyloid deposition is not known, classification by etiology is not possible; and since there is no constancy in the clinical picture, clinical considerations must be kept in the background. The simplest classification, taking into account the known factors, and not stressing hypothetic factors, would be on the basis of anatomic distribution. The following is suggested.

1. *Typical amyloidosis:* Deposition of amyloid in the usual sites (kidneys, spleen, liver, adrenal, etc.)
   a. Associated with other disease (as, tuberculosis, multiple myeloma, carcinoma, osteomyelitis)
   b. Not associated with other disease (rare, but occasionally reported as "primary")
2. *Atypical amyloidosis:* Amyloid, not following the usual or typical

distribution, found in one or many foci or organs, with or without symptoms

a. Associated with other disease or conditions (as, multiple myeloma, Hodgkin's disease, carcinoma, pyelonephritis, bronchiectasis, and the like)
b. Not associated with other disease (including most of the cases reported in the literature as primary amyloidosis, whether systemic or local)

For purposes of classification it is necessary to be somewhat arbitrary, and it is proposed that the parenchymatous involvement of liver, spleen, kidneys, and adrenals be considered *typical.* The addition of other organs (for example, the unusual cases of Gerber [17] and Edens [18]) should not be sufficient to remove it from this category. On the other hand, *atypical* should be applied to those cases in which liver, spleen, kidneys, and adrenals are spared, or in which only one of them is involved with extensive amyloid in less usual sites as defined by Lubarsch.[6] This is admittedly an arbitrary division, but the diversity of cases is so great that otherwise no simple schema is feasible.

The most widely accepted classification hitherto is that of Reimann, Koucky, and Eklund,[19] who defined four groups: Primary, secondary, amyloid with multiple myeloma, and tumor-forming amyloid. More logical is the classification of Rosenblum and Kirshbaum,[20] who divided amyloidosis into primary or idiopathic and secondary or symptomatic, with subdivisions in each group of diffuse or typical, and localized or atypical.

It is the contention of this paper that there is no warrant for the use of the terms primary and secondary. Lindsay [2] has already expressed objection to these terms, suggesting "that when the basic mechanism is known, primary amyloidosis will be classified as a 'secondary' type."

Amyloidosis, however, takes so many different forms that a few words of comment are in order. One of the most interesting groups is that associated with multiple myeloma. Of approximately 650 cases reported in the literature,[9, 21, 22] concomitant amyloidosis was noted in 41. Most often there is an atypical distribution, but sometimes, as far as can be determined from available data, the distribution is comparable to that seen after tuberculosis, designated typical in this paper. Reimann et al.[19] would call this group neither primary nor secondary, but would relegate it to a separate category. Similarly, so-called tumor-forming amyloidosis which may involve bones,[23, 24] upper respiratory tract,[25] or conjunctiva [26] need not have a separate niche apart from

other forms, but can be grouped with atypical amyloidosis. Some of these cases are associated with multiple myeloma,[21, 22] others show no associated disease. The classification proposed herein readily accommodates these cases. The skin disease lichen amyloidosis,[27-30] frequently reported in the dermatologic literature, is another example of atypical amyloidosis. There seems no need for separate categories or divisions for each of the many examples of localization of amyloid.

ATYPICAL AMYLOIDOSIS, ASSOCIATED WITH SENILITY

The first 5 cases reported in this paper appear to form a single group, all showing amyloid in the heart, with little or no localization elsewhere. The amount of amyloid was relatively small compared to that in some of the cases described in the literature,[2] and was found incidentally at autopsy. All of the patients were 80 years of age or over. They all showed other lesions adequate to explain death, and the only common feature was advanced age. These cases apparently are identical with the 3 reported by Ranström,[31] in patients 80, 81, and 88 years of age. The older literature contains a brief note by Beneke [32] reporting 6 similar cases in old individuals, but the ages are not given. The case of Beneke and Bönning [33] is probably in the same category, but again age and clinical data are not presented.

The conclusion seems justified, on the basis of my own and other cases, that the amyloid deposition in the heart without clinical symptoms, can be correlated with old age. It appears to be entirely misleading to consider such cases as primary. It is of interest that of the 5 cases, 4 were found in a period of 16 months, in a total of 193 autopsies. In this small series 19 patients were 80 years of age or over, giving a percentage of 21 for that age group. This suggests that the condition is far more prevalent than has hitherto been suspected.

There is independent evidence that under certain circumstances amyloid deposition is a function of age. In an investigation of amyloid in the genito-urinary tract, Bursell [34] studied deposition in the seminal vesicles and found an incidence increasing with age. Thus, in the age group 76 to 90, of 38 cases, amyloid was found in 13. Its deposition was not related to inflammation in the prostate. It is plausible that his cases belong in the same category of atypical amyloidosis associated with old age. In my own material, unfortunately, the seminal vesicles were not examined microscopically, but it is only a question of time until the correlation between the two groups is proved or disproved.

Why the advanced age group should show a significant amount of amyloid is not clear. It is possible, as suggested by Warren,[35] that atypical

amyloidosis represents a "widespread perversion of function" of the connective tissue, with dysfunction of fibroblasts. Possibly local ageing of fibroblasts may be a relevant factor. In this connection it is of interest to note the occasional finding of amyloid in sclerosed pancreatic islands of many diabetic subjects (atypical amyloidosis associated with diabetes), an observation indicating the importance of local factors.

ATYPICAL AMYLOIDOSIS ASSOCIATED WITH PYELONEPHRITIS

In the sixth case reported here, with a massive nephrolithiasis and severe pyelonephritis, the amyloid showed a curious localization. Its parenchymatous distribution was limited to the renal glomeruli, but in addition it was found in the walls of small blood vessels in many organs, including the heart. This distribution is unusual, and deserves the designation of atypical. We may assume that the concomitant pyelonephritis was the most significant associated disease. It is reasonable to designate this case as atypical amyloidosis associated with pyelonephritis. It is plausible that the infection, in this instance, had the same causal importance that tuberculosis or osteomyelitis has in typical amyloidosis. The peculiar localization is not explained. (The case suggests some similarities to that of Binford.[36])

Three important unsolved problems haunt the subject of amyloidosis: (1) Why does amyloid disease usually take a typical distribution, but occasionally an atypical localization? (2) What is the true proximal cause (or causes) of amyloid production (as contrasted with inciting or mediating factors or associations, such as the familiar tuberculosis)? (3) What, if any, are the essential chemical differences between the typical and atypical amyloid? It is probable that a thorough-going answer to any of these problems will automatically solve all of them.

Staining of Amyloid with Silver

Variations in the staining properties of amyloid have long been noted. The atypical form, especially, shows much inconstancy in respect to the usual tinctorial reactions. Hass and Schulz [37] have demonstrated the chemical complexity of the usual or atypical amyloid. Similar work has not been done with the atypical forms. Adequate chemical analysis may well display many differences. Meanwhile the tinctorial, or crudely histochemical studies must be relied on.

That amyloid can be impregnated with silver first came to my attention while studying with the late Dr. Pio del Río-Hortega. In the course of impregnating reticulin fibers of the spleen, some amyloid which was pres-

ent stained beautifully, in a tone different from either collagen or reticulin. This impregnation aroused no interest or special comment, and was accepted in his laboratory as a well known phenomenon. In the course of subsequent studies on silver impregnations of the nervous system, a simple method was adapted for the differential exhibition of senile plaques and Alzheimer strands.[38,39] This method, based on one of Hortega's nuclear stains, was found to impregnate amyloid with facility and clarity. A brief resumé of the method is given.

Thin frozen sections are washed in water, and placed in an ammoniacal silver solution. To 5 cc. of 10 per cent silver nitrate, there is added, dropwise, enough ammonia to produce and then just dissolve the characteristic precipitate. Then 6 to 8 cc. of sodium carbonate solution are added. Of crystalline sodium carbonate, I used a 5 per cent strength; of the anhydrous form, 3.5 per cent. Or, saturated lithium carbonate may be used as the added alkali. The resulting solution is diluted to 75 cc. with distilled water. To about 10 cc. of this solution, a few drops of pyridine are added and the solution gently heated in a small, covered beaker and lightly agitated until the sections turn a tobacco-brown. The temperature should not exceed about 45° C., which can be tested by applying the bottom of the beaker to the back of the hand. The brown sections are then washed in sodium thiosulfate ("hypo"), followed by water, and are mounted without toning. *No formaldehyde or other reducing agent is employed at any point.* The method gives indifferent results in paraffin sections, which can be kept in the silver solution in the paraffin oven until they turn a rich brown. This varies from ½ to 2 hours. The staining is far more diffuse than with frozen sections, and muscle cytoplasm is too deeply impregnated. Paraffin sections are not recommended.

Examples of frozen section impregnation with this method are shown in Figures 2, 3, 4, and 6. The amyloid stains deeply, but nuclei and lipochrome pigment also are deeply impregnated. Cytoplasm is frequently stained a light brown, and a few wisps of collagen may impregnate. Sometimes protein casts in the lumina of renal tubules stain with moderate intensity. Good silver impregnations were obtained in all 6 cases reported here. The results are permanent. Preparations from case 1 are vivid after a lapse of 6 years. In Figure 6 is shown an amyloid liver from a case of tuberculosis, to illustrate the staining of so-called secondary amyloid.

The method is more cumbersome than the methyl violet or Congo red reaction, and is not suggested as a substitute. It is presented as showing an interesting chemical property of amyloid, namely, an intense argyrophilia.

The reaction of amyloid with ammoniacal silver offers a new tool. The method is reported, not as a substitute for the simpler staining technics, but as a hitherto undescribed property of amyloid. It was previously shown in studies on senile brains [39] that the intracellular, Alzheimer strands as well as the interstitial senile plaques were strongly argyrophilic. That is, ammoniacal silver, facilitated by gentle heat, would impregnate these structures with regularity and intensity without

the use of any reducing agent. Nuclear material and lipochrome pigments also were regularly impregnated by the same method. Nerve fibrils and some connective tissue fibrils might be inconstantly and lightly stained. In the studies on amyloid the staining is not entirely selective, for, as can be seen in the illustrations, nuclei and lipochrome stain deeply. In general there is very good differentiation between amyloid and collagen, but occasionally some strands of heavy collagen will darken markedly with this technic. Further work is needed to elucidate the relationships and to correlate argyrophilia with other tinctorial properties.

SUMMARY

The present-day classification of amyloid disease, with its distinction of primary and secondary, involves the user in inevitable inconsistencies. A new classification is proposed, based on typical or atypical distribution, and on association or lack of association with other diseases or conditions. With this classification, a new category, "atypical amyloidosis associated with senility," is presented. Five such cases are described, in subjects over 80 years of age in whom the amyloid was present almost exclusively in the heart, and only as an incidental autopsy finding. A further case of a different category, atypical amyloidosis associated with pyelonephritis, also is presented.

A newly described chemical property of amyloid is its ability to combine with ammoniacal silver without the use of any reducing agent. A simple technic is described for the application of this method, which proves to be a useful tool for the discovery and identification of this substance.

REFERENCES

1. Koletsky, S., and Stecher, R. M. Primary systemic amyloidosis: involvement of cardiac valves, joints and bones, with pathologic fracture of the femur. *Arch. Path.*, 1939, **27**, 267–288.
2. Lindsay, S. The heart in primary systemic amyloidosis. *Am. Heart J.*, 1946, **32**, 419–437.
3. Perla, D., and Gross, H. Atypical amyloid disease. *Am. J. Path.*, 1935, **11**, 93–112.
4. Wade, L. J., Schlademan, K. R., and Steinberg, F. U. Amyloid disease of the kidney. *J. Missouri M. A.*, 1945, **42**, 335–338.
5. Huebschmann, P. Über Herzamyloid. *Virchows Arch. f. path. Anat.*, 1907, **187**, 35–46.
6. Lubarsch, O. Zur Kenntnis ungewöhnlicher Amyloidablagerungen. *Virchows Arch. f. path. Anat.*, 1929, **271**, 867–889.
7. Bannick, E. G., Berkman, J. M., and Beaver, D. C. Diffuse amyloidosis. *Arch. Int. Med.*, 1933, **51**, 978–990.
8. Schalong, H. Lymphogranulomatose und Amyloidose. *Virchows Arch. f. path Anat.*, 1925, **257**, 15–22.
9. Blumberg, N., and Fischbach, M. W. Plasmacytoma with amyloidosis. *M. Rec.*, 1945, **158**, 281–284.

10. Cabot case 31212. *New England J. Med.*, 1945, **232**, 632–637.
11. Levenson, S. M., Tagnon, H. J., Goodpastor, W. L., Green, R. W., Taylor, F. H. L., and Lund, C. C. Addison's disease associated with amyloidosis following thermal burns. *New England J. Med.*, 1947, **237**, 152–156.
12. Zemp, F. E. Primary amyloidosis: report of a case. *Ann. Int. Med.*, 1947, **26**, 448–453.
13. Soisalo, P., and Ritama, V. Zur atypischen Amyloidose mit besonderer Berücksichtigung des Herzens. *Acta med. Scandinav.*, 1943–44, **116**, 260–272.
14. Orloff, J., and Felder, L. Primary systemic amyloidosis: jaundice as a rare accompaniment. *Am. J. M. Sc.*, 1946, **212**, 275–279.
15. Dillon, J. A., and Evans, L. R. Primary amyloidosis: a report of three cases. *Ann. Int. Med.*, 1942, **17**, 722–731.
16. Lindsay, S., and Knorp, W. F. Primary systemic amyloidosis. *Arch. Path.*, 1945, **39**, 315–322.
17. Gerber, I. E. Amyloidosis of the bone marrow. *Arch. Path.*, 1934, **17**, 620–630.
18. Edens. Über lokales und allgemeines Amyloid. *Virchows Arch. f. path. Anat.*, 1906, **184**, 137–149.
19. Reimann, H. A., Koucky, R. F., and Eklund, C. M. Primary amyloidosis limited to tissue of mesodermal origin. *Am. J. Path.*, 1935, **11**, 977–988.
20. Rosenblum, A. H., and Kirshbaum, J. D. Multiple myelomas with tumor-like amyloidosis. *J. A. M. A.*, 1936, **106**, 988–991.
21. Atkinson, F. R. B. Multiple myelomata. *M. Press*, 1937, **195**, 312–317; 327–333.
22. Wells, A. H., and Goldish, D. R. Diagnostic case report. *Minnesota Med.*, 1945, **28**, 920–923.
23. Bürgi, U. Über einen Fall von solitärem Amyloidtumor des Scheitelbeins. *Frankfurt. Ztschr. f. Path.*, 1937, **50**, 410–428. (Abstract in *Arch. Path.*, 1939, **27**, 389.)
24. Bado, J. L., Brachetto Brian, D., and Cagnoli, H. A propósito de un caso de tumor amiloideo solitario de la extremidad superior del femur. *Bol. Soc. cir. d. Uruguay*, 1945, **16**, 84–107.
25. Kramer, R., and Som, M. L. Local tumor-like deposits of amyloid in the larynx. Report of a case with a review of the literature. *Arch. Otolaryng.*, 1935, **21**, 324–334.
26. Elles, N. B. Amyloid disease of the conjunctiva. *Am. J. Ophth.*, 1945, **28**, 486–497.
27. Greenbaum, S. S., and Bauer, J. T. Primary lichen amyloidosis. *Arch. Dermat. & Syph.*, 1947, **55**, 251–255.
28. Rocha, G. L. Líquen amilóide. *An. brasil. dermat. e sif.*, 1944, **19**, 211–216.
29. Malbran, C. F., and Bosq, P. Amiloidosis cutánea primitiva (líquen amilóidico). *Rev. argent. dermatosif.*, 1945, **29**, 33–36.
30. Dostrovsky, A., and Sagher, F. Localized amyloidosis of the skin. *Arch. Dermat. & Syph.*, 1941, **44**, 891–906.
31. Ranström, S. Amyloidosis myocardii. *Acta med. Scandinav.*, 1945–46, **123**, 111–125.
32. Beneke, R. Ueber lokale Amyloidose des Herzens. *Centralbl. f. allg. Path. u. path. Anat.*, 1922–23, **33**, 240–241.
33. Beneke, R., and Bönning, F. Ein Fall von lokaler Amyloidose des Herzens. *Beitr. z path. Anat. u.z. allg. Path.*, 1908, **44**, 362–385.

34. Bursell, S. Beitrag zur Kenntnis der Para-amyloidose im urogenitalen System unter besonderer Berücksichtigung der sog. senilen Amyloidose in den Samenbläschen und ihres Verhältnisses zum Samenbläschenpigment. *Upsala läkaref. förh.*, 1942, **47**, 313–328.

35. Warren, S. Generalized amyloidosis of the muscular systems. *Am. J. Path.*, 1930, **6**, 161–167.

36. Binford, C. H. Primary amyloid disease of the myocardium and blood vessels (report of a case with death from myocardial failure). *Arch. Path.*, 1940, **29**, 314–320.

37. Hass, G., and Schulz, R. Z. Amyloid. I. Methods of isolating amyloid from other tissue elements. *Arch. Path.*, 1940, **30**, 240–259.

38. King, L. S. The impregnation of neurofibrils. *Yale J. Biol. & Med.*, 1941–42, **14**, 59–68.

39. King, L. S. Pathology of senile brains. *Arch. Neurol. & Psychiat.*, 1942, **48**, 241–256.

[*Illustrations follow*]

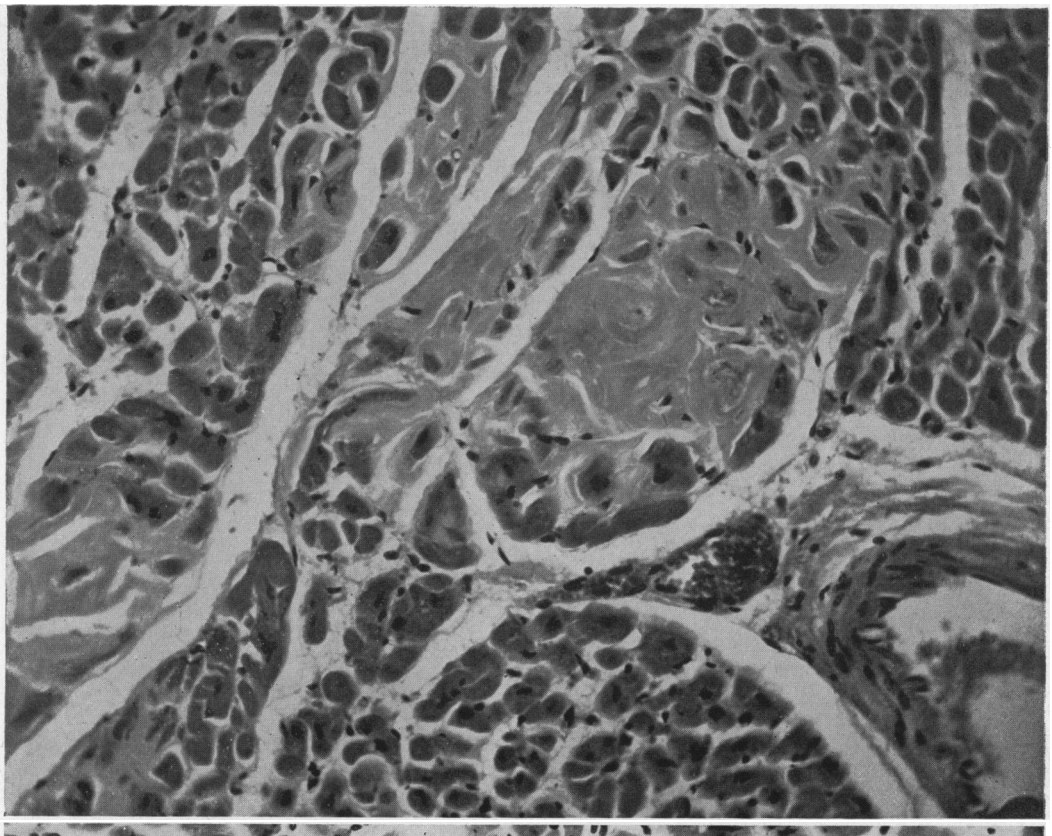
DESCRIPTION OF PLATES

PLATE 178

FIG. 1. Myocardium, case 4. Patches of amyloid occupy the interstitial tissue, replacing many muscle fibers and surrounding others in ring-like fashion. Many of the surrounded fibers are atrophic. Hematoxylin and eosin stain, paraffin section. × 600.

FIG. 2. Myocardium, case 4. The amyloid appears as dark-staining, lumpy masses surrounding some individual muscle fibers and replacing others. The interstitial connective tissue is essentially unstained, but nuclei and lipochrome pigment are impregnated. Silver impregnation, frozen section. × 600.

1



2

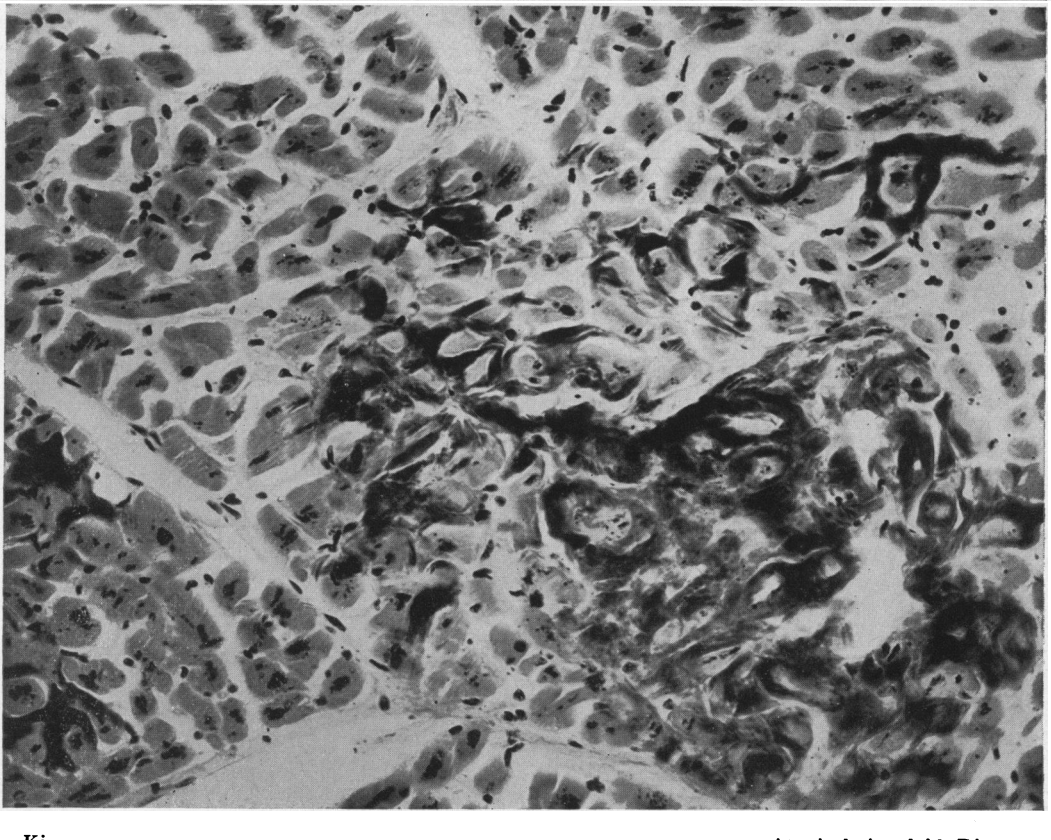


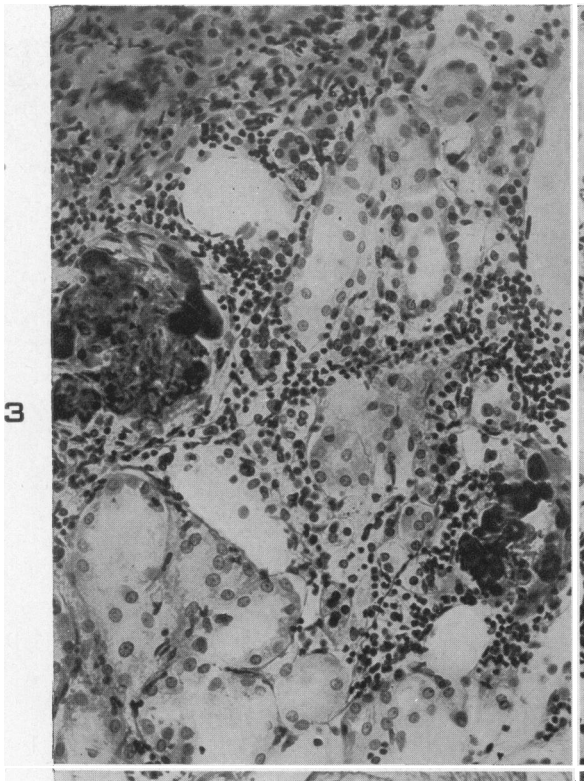
PLATE 179

FIG. 3. Kidney, case 6. Amyloid within the glomeruli is heavily stained, and contrasts well with the connective tissue of the thickened Bowman's capsules. Nuclei are well shown. Silver impregnation, frozen section. × 500.

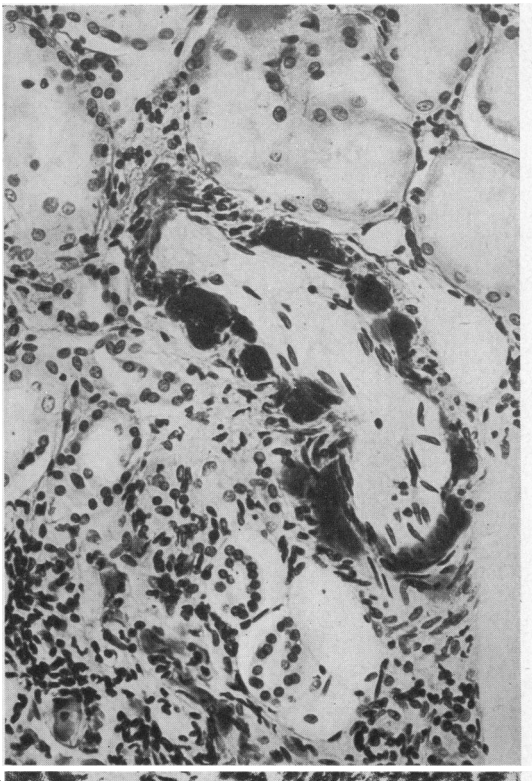
FIG. 4. Kidney, case 6. Amyloid is seen within the walls of a small blood vessel. Silver impregnation, frozen section. × 500.

FIG. 5. Adrenal, case 6. The amyloid in one of the periadrenal blood vessels is well shown. The general appearance after Congo red staining is similar to that seen with silver impregnation. No amyloid was present in the adrenal gland proper. Congo red and hematoxylin, paraffin section. × 500.

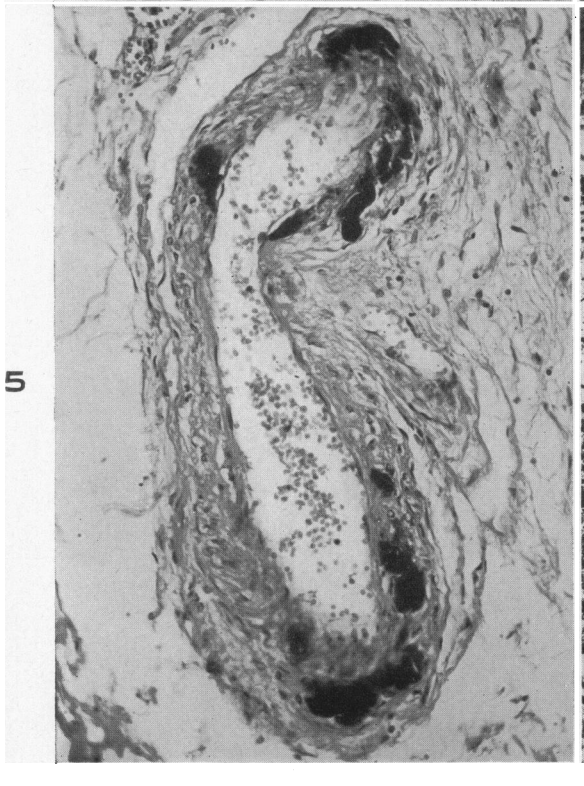
FIG. 6. Amyloid liver from a case of tuberculosis. In the material studied to date, there is no difference in reaction to silver between typical and atypical amyloidosis. At this magnification nuclei are visible as small dots. The extensive amyloid distribution is well shown. Silver impregnation, frozen section. × 55.



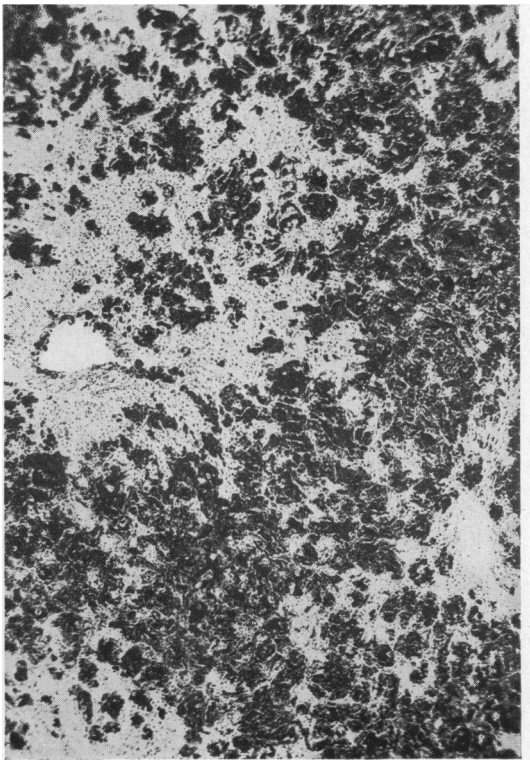
3



4



5



6