PRIMARY AMYLOIDOSIS: A REVIEW

BY

W. St. C. SYMMERS

From the Department of Morbid Anatomy, Charing Cross Hospital and Medical School, London

Über dem eigentlichen Wesen dieser sonderbaren Eiweissveränderungen und -ablagerungen liegt noch erhebliches Dunkel.—O. LUBARSCH.

This review is concerned mainly with "primary amyloidosis." It is necessary to stress at the outset that there are important misconceptions of what the term primary amyloidosis denotes, and that the problems of primary amyloidosis cannot profitably or realistically be separated from the problems of amyloidosis in general.

The term "primary amyloidosis" refers to those cases of amyloidosis in which no predisposing cause is found. Amyloidosis which occurs in association with a predisposing cause is now usually described as "secondary amyloidosis." The descriptions "primary" and "secondary" should not be interpreted as having definitive aetiological significance or as precluding an even closer relationship between these forms of amyloidosis than is yet apparent.

No other criterion than the absence of a recognizable predisposing cause is needed in order to designate any case of amyloidosis as primary amyloidosis. It is a common misconception that this diagnosis necessarily implies a particular and unusual distribution of amyloid, that the amyloid presents anomalous staining reactions, and that the disease has a characteristic clinical picture. On the other hand, it is true that the gross distribution of amyloid in most cases of primary amyloidosis differs remarkably from that in typical cases of secondary amyloidosis, that the staining reactions of the amyloid in primary and secondary amyloidosis may differ, and that in some cases of primary amyloidosis a foreign-body giant-cell reaction develops around the amyloid, or calcium is laid down in it: however, none of these characteristics is of absolute diagnostic significance. In fact, the varieties of amyloidosis cannot properly be defined in terms of the location, form, or tinctorial characteristics of the amyloid material, nor is there any clinical manifestation or syndrome associated with amyloidosis which occurs exclusively in one or another form of the disease. There are cases of primary amyloidosis which, except for the

absence of predisposing disease, do not differ, clinically or pathologically, from classical cases of secondary amyloidosis; conversely, clinical and pathological findings which are usually looked upon as typical of primary amyloidosis may be presented by cases of secondary amyloidosis.

Even the absence or presence of a recognizable predisposing cause is not a satisfactory criterion by which to differentiate varieties of amyloidosis. The recognized predisposing causes are gross disease processes: the commonest nowadays are chronic sepsis superimposed upon tuberculous lesions, particularly in the lungs, or associated with bronchiectasis; myelomatosis; and rheumatoid arthritis and spondylitis. Rarer predisposing causes include chronic infections of bones or joints, chronic ulcerative colitis, regional enteritis, Hodgkin's disease, bacterial endocarditis, and chronic infection of cancerous growths or of severe burns. When such overt diseases are present the association with amyloidosis is too obtrusive for the possibility of a causal relationship between the two to be overlooked. A relationship between less obvious diseases and amyloidosis may escape notice and the amyloidosis be mistakenly designated as primary. However, there is a group of cases in which amyloidosis occurs without any demonstrable predisposing affection: at present, the aetiology of this group is obscure.

The problems of amyloidosis call for the combined endeavour of clinicians, chemists, and pathologists, for many disciplines are challenged by our ignorance of the nature and pathogenesis of amyloidosis. Many cases, especially of primary amyloidosis, are still recognized too late for full investigation; indeed, until primary amyloidosis is more regularly considered in differential diagnosis many cases will be identified first at necropsy, or when post-mortem material is studied histologically.

It is of the first importance for the collection of detailed records of fully investigated cases that the clinician should be on the look-out for early cases of all forms of amyloid disease.

Historical Note

The condition which we know as amyloidosis appears to have been described first by Rokitsansky (1842), who noted the appearance of the liver, spleen, and kidneys, and mentioned the occurrence of "Bright's disease" when the kidneys were involved. Rokitsansky, who clearly was well acquainted with the disease and did not regard it as a rarity, noted its occurrence in association with "constitutional diseases of vegetation," including "scrofulosis, rachitismus, inveterate syphilis and quicksilver cachexia." Curiously enough, the first description of amyloidosis is often attributed to Virchow (1855a), or to Wilks (1856) or Budd (1857), although Virchow's own writings (1854a, b, c, 1855a, b) make it clear that amyloidosis was already quite well known at that time, when such terms as waxy degeneration, lard change, and sago spleen were already in use. In Britain, Budd, who was acquainted with Rokitsansky's work, had already described the "lardaceous" liver at some length in the first edition of his book (1845) on the liver, while "waxy degeneration" was the subject of a discussion in Edinburgh, in 1853, in which John Hughes Bennett, Sanders, and W. T. Gairdner took part (*Proceedings of the Physiological Society of Edinburgh, 1854*).

Virchow's interest in the condition seems to have developed during his search for substances in animal tissue which resembled cellulose or starch. Investigations of the colour reactions of various structures exposed to iodine and sulphuric acid showed him that only the so-called corpora amylacea of nervous tissue and the waxy-looking material in organs affected by what we now call amyloidosis gave reactions comparable to those of the vegetable substances. He concluded that the material of "corpora amylacea" did not have all the qualities of either starch or cellulose, but that it was probably isomeric with both and might be called by a name which had been used by botanists for starch-like substances—amyloid (Virchow, 1854c). In his subsequent work Virchow (1855a) used this term to describe the abnormal material present in viscera showing "waxy degeneration." He noted the association of this "amyloid degeneration" with chronic affections of bone, and that lymph nodes and the alimentary tract might be extensively involved in addition to the already well-known changes in the spleen, liver and kidneys:

he observed the early involvement of arteries in the affected parts, and suggested that amyloid was formed in the diseased bones and carried in solution in the blood to the sites of its deposition in the tissues.

Wilks (1856), whose account of "lardaceous disease" appeared shortly after Virchow's work, noted the association of amyloidosis with chronic suppuration, syphilis, tuberculosis, and "rheumatism." Recently, several authors have said that Wilks was the first to describe cases of primary amyloidosis: it is true that his series of cases included two in which there was no record of a predisposing disease (Cases 28 and 29), but it should be noted that he made a point of mentioning the possibility that bone disease might have been overlooked in these cases. Although one may find some support for a diagnosis of primary amyloidosis in Wilks's account of his Case 28 it is quite impossible to justify a definite retrospective diagnosis by the data available.

Wild (1886) is usually said to have been the first to recognize the occurrence of amyloidosis without any apparent predisposing disease. Wild himself, however, referred to the observations of Soyka (1876), who not only described the occurrence of such cases in both young and old patients, but also noted the predominant involvement of the heart in the latter. Primary generalized amyloidosis did not begin to become widely known until Lubarsch (1929) described three more cases, drew attention to a small number of cases recorded since Wild's communication, and defined the features which he considered to distinguish this form of amyloidosis from secondary amyloidosis. Strauss (1933) collected 28 cases from the literature. The first major account in English was by Koletsky and Stecher (1939), who reviewed 24 cases; this number was brought up to 48 by Eisen (1946), to 71 by Higgins and Higgins (1950), and to 98 by Mathews (1954), whose review is particularly valuable. Some cases of primary generalized amyloidosis which were overlooked by these authors, and others published recently, bring the total to over 150. This number would be considerably increased if cases of primary amyloidosis in old age were included. This so-called senile amyloidosis is common, and affects the heart predominantly, as Soyka (1876) observed: Hüsselmann (1955) analysed no fewer than 76 personally studied cases, and his investigations suggest that about 15% of men and 10% of women over 70 have cardiac amyloidosis.

It may be assumed that many cases of primary amyloidosis are not published. From random

enquiries made to about a dozen colleagues in England I have heard of over 20 unreported recent cases, excluding senile amyloidosis. In 10 years I have personally seen 10 cases among about 4,000 necropsies in general hospitals: in this material 10 cases of secondary amyloidosis were also found, including two cases associated with myelomatosis.

Changing Trends in the Incidence of Amyloidosis

Until comparatively recently most cases of amyloidosis were of the secondary type. Preventive medicine and diagnostic and therapeutic advances have now brought about such a reduction in the severity, chronicity, and incidence of chronic sepsis, tuberculosis, and syphilis that secondary amyloidosis in general has become comparatively uncommon, and a higher proportion of cases of secondary amyloidosis is now associated with what were formerly numerically insignificant predisposing causes, such as myelomatosis, rheumatoid disease, and infected cancers. The apparently true rise in the incidence of myelomatosis and the less certain rise in that of rheumatoid arthritis have played only a small part in the change which has taken place in the relative frequency of the various predisposing causes of secondary amyloidosis. The overall decline in the frequency of secondary amyloidosis has considerably raised the proportion of cases which are of the primary type. It is quite possible, although by no means certain, that there is an actual increase in the incidence of primary generalized amyloidosis: the increasing number of cases recognized is undoubtedly due in part to greater awareness of the occurrence of the disease, but there is a growing impression that the number is genuinely larger than may be explained simply on these grounds.

These changing trends in the incidence of amyloidosis reflect a wider change in human pathology, as advances in diagnosis and therapeutics and in preventive medicine alter the incidence and course of many diseases, and bring fresh problems dependent on a lengthened life-span or arising from the undesirable side-effects of new therapeutic agents.

Where in the past the clinical importance of amyloidosis lay in its occurrence as a complication of other chronic diseases, it is now coming progressively more to the attention of diagnosticians as a morbid process in its own right. Quite apart from those cases which present with mani-

festations directly caused by generalized amyloidosis, various local manifestations make amyloidosis an important condition in the differential diagnosis of many common diseases: the simulation of peptic ulcer or carcinoma by gastric amyloidosis, of urinary disorders by vesical amyloidosis, of bronchopulmonary disorders by respiratory-tract amyloidosis, and of neuritis by amyloid neuropathy are noteworthy examples, to which many others could be added. Amyloidosis as a cause of intractable heart failure or of a protean variety of skin lesions merits special mention. It is ironical that amyloidosis, once almost as often a consequence of syphilis as of any other disease, should be taking over the role of syphilis as the mimic among diseases.

Classification of Amyloidosis

A rational classification of amyloidosis will not be possible until we know a great deal more than at present about the aetiology and pathogenesis of the various forms of amyloidosis and their relation to immunological responses and to metabolism of proteins and other substances. Three classifications which are commonly referred to in the literature may be noted here.

A. Clinicopathological Classification.—Reimann, Koucky, and Eklund (1935) proposed a clinicopathological classification, namely:

1. *Primary Amyloidosis.*—In this form the condition is not associated with an obvious predisposing disease. Organs and tissues are predominantly affected which are not ordinarily involved in secondary amyloidosis; the amyloid tends to stain atypically; nodular deposits of amyloid may occur.

2. *Secondary Amyloidosis.*—In this form the condition is associated with a recognized predisposing disease (other than myelomatosis). The liver, spleen, kidneys, and adrenals are predominantly involved; the amyloid stains typically.

3. *Tumour-forming Amyloidosis.*—In this form solitary or multiple tumour-like masses of amyloid occur in various situations.

4. *Amyloidosis Associated with Myelomatosis.*

B. Anatomical Classification.—King's (1948) classification was based on the anatomical distribution of the amyloid deposits thus:

1. *Typical Amyloidosis*, with deposition of amyloid in the classical sites:

- (a) Associated with other diseases
- (b) Not associated with other diseases

2. *Atypical Amyloidosis*, with predominant deposition of amyloid in sites other than those involved in the classical type :

- (a) Associated with other diseases
- (b) Not associated with other diseases

C. Dahlin's Classification.—Dahlin (1950) classified amyloidosis into :

1. *Primary Amyloidosis*
 - (a) Systemic
 - (b) Focal or tumefactive
2. *Amyloidosis Complicating Myeloma*
 - (a) Systemic
 - (b) Focal or tumefactive
3. *Secondary Amyloidosis*

The classification of Reimann *et al.* (1935) was introduced only six years after Lubarsch (1929) had focused attention on primary amyloidosis ; the unreliability of the distribution and staining reactions of amyloid as criteria for classification had not yet been appreciated. Dahlin's (1950) classification, like that of Reimann *et al.* (1935), gave the status of a separate entity to amyloidosis occurring in association with myelomatosis ; although it is true that the amyloidosis of myelomatosis usually shows a distribution similar to that of primary amyloidosis its classification in a category distinct from amyloidosis secondary to other diseases does not appear to be necessary. There is less to criticize in King's (1948) classification, although it does not provide for those cases, which may occur with or without a predisposing disease, in which there is virtually equal involvement of the organs usually affected in typical cases of secondary amyloidosis and of the organs and tissues which are characteristically affected in primary amyloidosis.

For practical purposes, and until further knowledge allows of a definitive classification, it seems best to classify amyloidosis on a clinicopathological basis: (1) Generalized amyloidosis associated with a recognized predisposing disease (generalized secondary amyloidosis) ; (2) Generalized amyloidosis in the absence of any recognized predisposing disease (generalized primary amyloidosis) ; (3) Localized amyloidosis. In this system of classification it should be noted that most cases of senile (predominantly cardiac) amyloidosis would come in the second category ; amyloidosis associated with myelomatosis comes in the first category. Genuine cases of amyloidosis localized to one site or to a single organ, e.g., purely cutaneous amyloidosis, some instances of respiratory-tract amyloidosis, and a few others, fall into the third category ; many cases of seemingly localized

amyloidosis prove to be instances of generalized amyloidosis (primary or secondary).

Synonyms

Primary amyloidosis goes under a strange variety of names, including atypical, primary atypical, typical atypical, unusual, idiopathic, essential, causeless, primary systemic, systematized, diffuse, generalized, juvenile, presenile, senile, vascular mesodermal, mesenchymal, nonvisceral, extra-abdominal false, and malignant amyloidosis, paramyloidosis, and Wild's disease or Wild-Lubarsch disease.

Morbid Anatomy and Histology of Primary Amyloidosis

Frequency of Involvement of Different Parts.—

It is difficult to estimate the frequency of amyloid deposits in different organs and tissues. Analysis of published accounts show that the thoroughness with which the pathological findings have been investigated or reported differs greatly from case to case: the data bearing on the distribution of amyloid are rarely comparable. The figures in the following table represent with reasonable accuracy the *minimum* percentage incidence of histologically demonstrated involvement of the tissues named, as found on analysis of 130 published cases of primary amyloidosis (excluding senile amyloidosis) and 15 personally studied cases. (As all the tissues listed were not examined in every case the stated percentage is likely to be lower than the real incidence.) Many other tissues have been examined in individual cases (Mathews, 1954), but the number of investigations is too small to warrant their inclusion in the table.

TABLE

Organ or Tissue	Minimum Percentage Incidence in 145 Cases
Heart	90
Alimentary tract	70
Tongue	40
Spleen	40
Liver	35
Kidneys	35
Lungs	30
Adrenals	25
Lymph nodes	20
Skeletal muscle	20

The following account of the gross and microscopical findings in primary amyloidosis is based partly on a survey of the literature and partly on the study of personal cases ; some of these cases are reported elsewhere (Symmers, 1956b).

General Observations on Morbid Anatomical Findings.—In a small proportion of cases of

primary amyloidosis there are no macroscopical abnormalities specifically indicating the diagnosis, which in such cases is first made histologically. In some cases there is extensive visceral amyloidosis, which is unmistakable to the observer who is acquainted with the classical appearance and feel of amyloid. In other cases amyloid deposits are visible but not obtrusive, and are likely to be overlooked if one is not on the watch for them. Even minute deposits, e.g., those beneath the endocardium, have the curious, translucent, grey or faintly pink or yellowish appearance and dull or matt surface which are characteristic of amyloid. It should be noted that the gross iodine and sulphuric acid test for amyloid is more unreliable in primary amyloidosis than in the classical secondary type.

General Observations on Histological Findings.

—The earliest lesions are minute deposits of amyloid in the interstitial tissues, usually in close association with reticulin fibres or on the surface of muscle cells or fat cells. The actual site of deposition of the amyloid is in dispute. Larsen (1930) maintained that amyloid is laid down originally beneath the endothelium of capillaries and spreads thence into the rest of the tissues. The ubiquity of capillaries makes it difficult to discount this interpretation: thus Larsen explained deposits within the walls of larger blood vessels as the result of involvement of vasa vasorum; however, there seems to be no doubt that amyloid deposits may form at a distance from endothelium, e.g., within the media of small arteries which appear not to be provided with vasa vasorum. The view which Peters (1943) advocated is more consonant with observed facts: according to Peters, amyloid is deposited initially in or on cell membranes, giving rise to such characteristic features of the histology of primary amyloidosis as the amyloid rings around fat cells and the investment of muscle cells by amyloid (Figs. 1 and 2). However, Dahlin (1949), among others, by combining silver impregnation of reticulin with metachromatic staining of amyloid by methyl violet, showed that in some tissues, e.g., liver, adrenal, and myocardium, the amyloid is intimately applied to reticulin fibres. As the condition progresses the amyloid deposits increase in extent

FIG. 1

FIG. 2

All the illustrations accompanying this paper have been prepared from cases of primary amyloidosis.

FIG. 1.—Pericellular amyloidosis of adipose tissue ("amyloid rings"). An area of unaffected tissue is included. Haematoxylin and eosin. $\times 140$.

FIG. 2.—Pericellular amyloidosis of myocardium. Congo red and haematoxylin. $\times 375$.

FIG. 3.—Severe myocardial amyloidosis, showing appearances as if the muscle cells merged into the amyloid material. Haematoxylin and eosin. $\times 110$.

FIG. 4.—Multiple amyloid foci (pale areas) in wall of pulmonary artery; the elastica is breached by some of the deposits. Verhoeff and Van Gieson. $\times 40$.

FIG. 5.—Juxta-arterial amyloid deposits with giant-cell reaction. Haematoxylin and eosin. $\times 180$.

and the cells of the affected tissues undergo atrophy through pressure and inanition, and eventually even the fibrous framework disappears without trace (Fig. 3). Compression atrophy of cells in areas of amyloidosis is most marked in parenchymatous organs, such as the liver, and in muscle tissue; in heavily affected areas of adipose tissue, however, the cells appear to die without appreciable alteration in size, and the appearances resemble a fresh ischaemic infarct, except for the distinctive pericellular amyloid rings. Often one is struck by the normal appearance of cells which are completely invested by amyloid: presumably metabolic exchanges are not seriously hampered until the increasing thickness or altered permeability of the amyloid layer makes it an effective barrier. Parenchymatous damage appears to result essentially from gradually progressive ischaemia associated with the increasing bulk of the amyloid deposits.

The amyloid forms, particularly around capillaries, around the muscle cells in the media (Fig. 4) of larger blood vessels (especially arterioles and arteries) in the musculature of hollow organs, in skeletal muscle, and in adipose tissue. Sometimes amyloidosis of blood vessels takes the form of irregular clumps in the connective tissue outside the media, and there is little or no spread

into the media: this juxtavascular form of amyloidosis may occur alone or in association with the commoner intramural vascular deposits. When a giant-cell "foreign-body" reaction develops around amyloid it usually occurs in association with these juxtavascular deposits (Fig. 5); the giant-cell reaction is uncommon, but it is less infrequent in amyloidosis complicating myelomatosis than in other cases.

In solid organs, the parenchyma may be gradually replaced by amyloid deposits, and the picture in such organs as the liver, kidneys, and spleen is then the familiar one seen in secondary amyloidosis; when their parenchyma escapes involvement, amyloid is confined to the walls of blood vessels or to capsule and trabeculae.

The predominant involvement of connective-tissue structures in primary amyloidosis, in contrast to the predominant parenchymatous in-

FIG. 6.—Amyloidosis of two blood vessels in capsule of liver. Haematoxylin and eosin. $\times 130$.

volvement in classical secondary amyloidosis, accounts for the use by some writers of the terms "mesodermal" or "mesenchymal" amyloidosis to distinguish this condition from "parenchymal" (secondary) amyloidosis. The distinction is an artificial one.

Affected blood vessels are eventually transformed into thick-walled amyloid cylinders, the endothelial lining and lumen ordinarily remaining intact (Fig. 6): elastic fibres are commonly the last component to disappear. Complete obliteration of the vessels is unusual, although sometimes amyloid cushions project into the lumen, particularly in the smallest vessels. Ischaemic changes in tissues supplied by affected arteries are not as uncommon as has sometimes been said; they occur oftenest in the myocardium and usually are manifested by focal cytolysis. Rarely, gross infarction occurs, usually because of thrombosis occurring on amyloid plaques involving the intima of larger vessels such as the coronaries; thrombosis is in general an uncommon complication of vascular amyloidosis.

Intracellular Amyloid.—In addition to the usual interstitial deposits, amyloid may be found within parenchymatous cells, particularly in the myocardium, skeletal muscle, kidney, and liver. Amyloid in the epithelial cells of renal tubules is usually situated between the nucleus and the base of the cell, and affected cells may become fused together. In muscle, amyloid is seen as a fine linear deposit in relation to the myofibrils; these early deposits may fuse to form considerable masses, distending the affected cells. Intracellular amyloid is comparatively rare, and its significance is uncertain (Reimann, Sahyoun, and Chaglassian, 1954). It has been suggested that dying cells become permeable to amyloid (Schmidt, 1904); it is possible that amyloid may form within viable cells, following absorption of a soluble precursor in tissue fluids or, in the case of the renal tubular epithelium, in the glomerular filtrate. Amyloid in the cytoplasm of macrophages, as may be seen particularly in lymph nodes, is probably merely a manifestation of phagocytosis. Whatever its explanation, the occurrence of intracellular amyloid seems to be only incidental in the development of the disease and not a manifestation of a fundamental pathogenetic mechanism.

Amyloid Nodules and Amyloid Striae.—Amyloid nodules (Fig. 7), which are a common macroscopical feature of primary amyloidosis, may originate as obliterative lesions affecting very localized stretches of small blood vessels, or as interstitial deposits unrelated to vessels. The

nodules appear to enlarge by the formation of more amyloid on their surface and occasionally by coalescence with adjacent deposits. Amyloid striae (Fig. 8) are a curious and unexplained feature of some cases: they present as parallel streaks of amyloid beneath serous or endothelial membranes, e.g., in the pericardium and endocardium. The striae may extend deeply into the underlying tissues: in the atria and intestine they may even cut through all layers of the wall, almost from surface to surface, irrespective of the natural planes of the tissues traversed.

Microscopical Appearances of Amyloid.—The amyloid material in cases of primary amyloidosis has the same amorphous and more or less homogeneous appearance in sections as the amyloid of secondary amyloidosis. As in the latter, the deposits often show an irregular patterning of ill-defined darker and paler areas, particularly in intramural vascular lesions. The interstitial nodules may have a laminated structure (Fig. 9). Irregular shrinkage during fixation and dehydration of the tissues may account for some of these variations in appearance, or they may represent different stages in "maturation" of the amyloid.

Amyloid deposits, particularly in the alveolar septa of the lungs, and in bones and kidneys, may undergo calcification. Ossification can also occur. Crystalline or crystalloid deposits of amyloid, such as are seen occasionally in myelomatous tumours, often with a giant-cell granulomatous reaction around the deposits (Ranström, 1951), have not been described in primary amyloidosis.

Staining Reactions.—Although amyloid is usually described as an eosinophil material its colour in haematoxylin-eosin preparations can depend on the haematoxylin used: Ehrlich's haematoxylin and similar muciphilic haematoxylin may reveal some degree of haematoxyphilia of the amyloid in some cases. In any type of amyloidosis the amyloid in sections may be coloured brownish by iodine, or a bluish or reddish grey by iodine followed by sulphuric acid, or there may be no reaction. Metachromatic staining with the rosanilin group of basic dyes, such as methyl violet and cresyl violet, may not be demonstrable in some cases, and is oftener lacking in primary amyloidosis than in the secondary type. When present, metachromasia may vary in intensity in different deposits, or in different parts of individual deposits. Similarly, affinity for Congo red varies from case to case, and maybe from place to place, and may be absent. The development of birefringence in amyloid on staining with Congo red has been observed, but is an

FIG. 7.—Multiple amyloid nodules in myocardium. There are also patches of pericellular amyloidosis (see Fig. 10), interstitial fibrosis, and myocardial cytolysis. Haematoxylin and eosin. $\times 30$.

FIG. 7

FIG. 8.—Partial transection of wall of heart by early amyloid striae (dark areas). Haematoxylin and eosin. $\times 15$.

FIG. 8

FIG. 9.—Small interstitial amyloid nodule in myocardium, showing laminated appearance. Haematoxylin and eosin. $\times 140$.

inconstant finding and of no diagnostic value. Prolonged preservation of amyloid tissue in formalin may lessen or abolish its specific staining reactions.

Amyloid often shows little or no reaction in periodic-acid-Schiff preparations; in some cases an intense positive reaction is obtained.

Frozen sections show abundant finely dispersed Sudanophil lipid in some amyloid deposits.

Trichrome stains of the Mallory and Masson types give inconstant results: sometimes amyloid deposits which appear homogeneous in other preparations persistently show circumscribed areas stained by the red acid dye and surrounded by areas which have been decolorized by phosphomolybdic acid and stained by the contrasting dye; the significance of this finding is obscure.

Phosphotungstic-acid-haematoxylin occasionally shows blue, fibrin-like material within amyloid deposits, but usually colours them brick-red.

Silver methods for staining amyloid are generally unreliable. Silver impregnation of reticulin followed by staining with methyl violet is sometimes useful for demonstrating early amyloid deposits.

Without doubt a well-done Van Gieson stain is one of the most useful stains for studying

amyloidosis, particularly in cases in which the amyloid shows little or no metachromasia with the rosanilin dyes. Even in tissues which contain a high proportion of picriphil material, such as muscle, Van Gieson's fluid demonstrates amyloid distinctly by the curious khaki colour which it usually develops; some amyloid deposits show a remarkable, pale flesh-pink colour. Van Gieson's fluid is particularly valuable for distinguishing between amyloid and hyalinized collagenous tissue, which, in spite of some histologists' claims to the contrary, cannot regularly be told apart in haematoxylin-eosin preparations. In relation to the differentiation of amyloid from hyalinized collagen it should be remembered that the latter may have appreciably greater affinity than normal collagen for Congo red.

Visceral and Other Lesions of Primary Amyloidosis.—In the following paragraphs the findings in the organs which are oftenest affected in primary amyloidosis are described systematically. Remote consequences of amyloidosis, such as chronic venous congestion, anasarca, and the like, require no further mention.

Heart.—Involvement of the heart (Figs. 10 and 11) is particularly frequent and important, and is directly responsible for death in most cases. The heart is usually enlarged, commonly weighing between 500 and 600 g. An infrequent observation, but one which is very suggestive of the diagnosis, is an extraordinary waxy rigidity and much diminished elasticity of the myocardium, so that it retains its shape when incised, almost as though it had been fixed in formalin. The myocardium is usually the most heavily affected part, often without predilection for atria or ventricles or for one side or the other; in some cases the atria are predominantly involved. The amyloid in the myocardium appears as translucent greyish, or occasionally yellowish, streaks or patches which may be barely visible or so extensive as to seem almost to replace the whole muscle; amyloid nodules, which may be minute or as large as 2 cm. in diameter, may also be found. Myocardial deposits are often continuous with similar deposits in the epicardium or endocardium. The epicardium, or both layers of the pericardium, may be sparsely or extensively studded with flattened or rounded subserosal nodules of amyloid, ranging in size from those just visible to the naked eye up to lesions 2 or 3 mm. in diameter; or there may be smooth or furrowed plaques of great or small extent, or a curious pattern of parallel sinuous streaks of amyloid, particularly in the pericardial sinus. Lesions like those in the peri-

FIG. 10.—Higher magnification of part of Fig. 7, showing confluent pericellular amyloidosis of myocardium. Haematoxylin and eosin. $\times 140$.

FIG. 11.—Amyloid deposits in subendocardial tissue. Haematoxylin and eosin. $\times 115$.

FIG. 10

FIG. 11

cardium may be found in the endocardium lining the chambers of the heart. The valves are usually normal, but in a considerable number of cases amyloid may be present in the form of small nodules, particularly on the ventricular aspect of the atrioventricular valves near the free margin of the cusps and at their base. In some cases part or the whole of one or more cusps may be uniformly thickened by a subendothelial plaque of amyloid up to 2 or 3 mm. in thickness. These lesions usually seem not to interfere significantly with the function of the valves; sometimes stenosis results, or nodules on the opposed surfaces of the cusps prevent proper closure. The mitral and tricuspid valves are affected oftener than the aortic or pulmonary valves; any valve may be involved alone.

The cardiac manifestations of primary generalized amyloidosis as described here do not differ from those of the predominantly cardiac amyloidosis of old age (primary "senile" amyloidosis), in which the degree of involvement may be just as variable and extreme as in the generalized form, of which it is probably a variant.

Alimentary Tract.—The oesophagus, stomach, and intestines are very commonly involved in cases of primary amyloidosis although clinical manifestations rarely result even when there are extensive lesions. Obstruction, ulceration, or haemorrhage may occur, particularly in cases of gastric amyloidosis, which have recently been reviewed by Cooley (1953). Pyloric obstruction has been noted in several cases of primary amyloidosis, and by Shnider and Burka (1955) is gastric amyloidosis complicating myelomatosis. Intestinal obstruction is rare; it occurred in Randall's (1933) case of amyloidosis with myelomatosis. The severer lesions of the alimentary tract take the form of bands or plaque-like masses of amyloid in the muscle coats, or even occupying the whole thickness of the wall; the affected length may be converted into an almost rigid tube, with walls up to a centimetre or more in thickness. In the earlier stages of gastrointestinal involvement the amyloid is commonly laid down in two situations: in and around the walls of the small blood vessels, and between the muscle fibres of the muscularis mucosae and of the main muscle coats. The amyloid is not uniformly distributed, vessels and musculature in some parts remaining unaffected when other parts are severely altered. Heavy involvement is followed by atrophy of the mucosa, which, with the associated ischaemia, predisposes to erosion or deeper ulceration; in the stomach the ulceration may be of peptic type.

Subserosal deposits, like those in the pericardium, may be present on the surface of the stomach and intestines or more widely throughout the peritoneal cavity.

Adipose Tissue.—In some cases the mesenteries and omenta, like adipose tissue elsewhere, may be extensively converted into firm, rubbery masses. There may be foci of fat necrosis in these areas, probably resulting from ischaemia; these present macroscopically as opaque white or yellow flecks or nodules which have the characteristic microscopical appearance of fat necrosis. As in any adipose tissue affected by amyloidosis the fat cells may be outlined by a distinct fine ring of amyloid, as described particularly by Peters (1943); occasionally the individual cells may be converted into solid amyloid masses. Rings of similar appearance may be seen on occasion around fat cells in disseminated lupus erythematosus; in haematoxylin-eosin preparations the appearances may indeed be identical with those in amyloidosis, although the two can readily be distinguished if the amyloid stains metachromatically or shows affinity for Congo red. In some cases only the evidence of amyloidosis in other tissues will indicate the nature of the change in the adipose tissue. In disseminated lupus erythematosus this appearance is due to fibrinoid material forming in the connective-tissue matrix in the interstices between the fat cells, and not to amyloid deposition. It is worth noting that a somewhat similar appearance may develop as a post-mortem change in adipose tissue exposed to proteolytic and lipolytic enzymes, e.g., in the vicinity of the pancreas.

Tongue.—Macroglossia has been described as an important diagnostic feature of primary amyloidosis, but it is by no means constantly present. It may also occur in amyloidosis secondary to any of the recognized causes. The enlargement of the tongue results from widespread deposition of amyloid between the muscle fibres (Fig. 12), which gradually disappear. The tongue may become so big that it fills the mouth, moulding itself to the palatal arch and teeth; sometimes the patient can no longer close his mouth, and in such cases oral infection, e.g., moniliasis, and ulceration may become troublesome and lead to septic pneumonia or gross inanition. The surface of the amyloid tongue is usually smoothed out, red and shiny. If there are superficial amyloid deposits the tongue may have a nodular or furrowed surface; amyloidosis is one of the causes of the curious condition known as "scrotal tongue."

FIG. 12.—Heavy amyloidosis of tongue, with compression and atrophy of muscle fibres. Methyl violet. $\times 55$.

FIG. 13.—Amyloidosis of pancreatic lobule. Haematoxylin and eosin. $\times 130$.

FIG. 12

Liver.—In contrast to its usual severe involvement in secondary amyloidosis, the liver may be macroscopically quite normal in primary amyloidosis, apart from such non-specific changes as fatty change or chronic venous congestion. It is un-

usual for careful histological examination not to show amyloid in some part of the liver: it may be confined to small branches of the arteries and to a few portal tracts. When the parenchyma is involved the appearances do not differ from those of secondary amyloidosis of corresponding severity. Occasionally liver disease dominates the clinical picture (Wollaege, 1950). Portal hypertension may develop with all its consequences, including haemorrhage from oesophageal varices (Pocock and Dickens, 1953).

FIG. 13

The *gall bladder* may show changes comparable with those in the stomach and intestines. Curiously, however, the muscle of the gall bladder and biliary passages seems to be seldom affected by amyloidosis, a relative immunity which it has in common with tracheobronchial muscle, and which contrasts with the high incidence of

amyloidosis of the musculature of the alimentary and urinary tracts.

Pancreas (Fig. 13).—Pancreatic involvement is common. Parenchymatous replacement by amyloid may be extensive and diabetes mellitus has been attributed to destruction of the islets in such cases (Pocock and Dickens, 1953).

Spleen.—The spleen may be macroscopically normal, or it may show focal or diffuse amyloid change, exactly as in classical cases of secondary amyloidosis. Microscopically, the appearances correspond with the gross findings; amyloid may usually be found in blood-vessel walls or in the connective tissue of the capsule and trabeculae even in cases with no naked-eye abnormality. Fatal spontaneous rupture of the spleen in primary amyloidosis has been recorded (King and Oppenheimer, 1948; Drapiewski, Sternlieb, and Jones, 1955).

Lymph Nodes.—Generalized lymph-node enlargement may result from amyloid deposition, or involvement may be restricted to one or two groups or even to isolated nodes; often the nodes are macroscopically normal. Amyloid involvement of lymph nodes appears to occur independently of the distribution of amyloid in other parts. Lymph nodes draining organs which are heavily affected by amyloidosis may be uninvolved, and conversely massive amyloid deposits may be present in nodes when there is little or no amyloid in the tissues which they drain. In most cases the amyloid appears first on the reticulin framework of the cortex and medulla of the nodes, with or without involvement of the follicles; ultimately the nodes may be converted into an almost solid mass of amyloid, or, oftener, the sinuses remain widely patent as a series of anastomosing channels between thick cords of amyloid which form the bulk of the node. Sometimes the follicles, alone of the lymphoid tissue, remain relatively intact, although surrounded by the amyloid masses. In some cases the amyloid deposits begin in the walls of the sinuses and spread thence into the lymphoid tissue; in these cases amyloid may be seen in the cytoplasm of swollen macrophages lining the sinuses. Severely affected nodes may be considerably enlarged, measuring up to 5 cm. or so in their longest dimension; usually the enlargement is less, and the nodes seldom exceed 2.5 cm. from pole to pole.

Kidneys.—When the kidneys are severely involved in primary amyloidosis the macroscopical and microscopical features are ordinarily the same as in classical secondary amyloidosis. Rarely, the

kidneys are somewhat shrunken. In a considerable proportion of cases there is no gross abnormality, or amyloid glomeruli may be just recognizable, with the help of a hand-lens, as minute translucent bodies. Histological study rarely fails to show some degree of involvement, whether confined to arterial walls or to patchy amyloidosis of occasional glomeruli. In some cases, particularly those of severer grade, amyloid may be present within the tubular epithelial cells as well as in the more usual locations. Amyloid casts may be found within some tubules. Multinucleated syncytial masses may form around amyloid in the tubules and in the interstitial tissue, even when a giant-cell reaction is not found in other tissues in the same case; the histological picture in such cases may resemble that of "myeloma kidney."

Amyloid deposits may convert parts of the *ureters* into rigid tubes (Higbee and Millett, 1956), and localized or extensive thickenings, folds or tumour-like masses of amyloid may be found in the *urinary bladder*. Atrophy of the covering epithelium may result, and infective cystitis is likely to develop, especially in women. Painless haematuria may be a feature of such cases clinically, and usually the source of the haemorrhage cannot be found. The vesical manifestations of primary generalized amyloidosis do not differ from those which may be found in cases of localized amyloidosis in which only the bladder appears to be affected (Beames, 1955).

Reproductive Organs.—The reproductive organs may be more or less extensively involved, but changes are rarely obtrusive. When extravascular genital amyloidosis is found it is usually in the form of deposits between the smooth muscle cells of the uterine tubes, myometrium, vasa deferentia, seminal vesicles and prostate (McDonald and Heckel, 1956). As in classical secondary amyloidosis and senile amyloidosis, the seminal vesicles may be particularly heavily involved. In the testes amyloid may be laid down on the basement membrane of the seminiferous tubules in such a way as to simulate, in haematoxylin-eosin preparations, the hyalinization of simple atrophy.

Respiratory System (Figs. 14 and 15).—Any part of the respiratory system may be severely affected by amyloid deposits in primary amyloidosis, and this involvement may dominate the whole clinical picture. In many cases, however, the respiratory system is macroscopically unaffected, and microscopical changes, if present, are confined to amyloid deposition in the walls of some blood vessels or in alveolar septa in scattered areas. Amyloid may be found, particularly in relation

to cartilage, in the mucosa of any part of the respiratory passages, including the nasal sinuses. The commonest site for nodular lesions is the larynx. Identical "amyloid tumours" may occur here in the absence of clinically detectable amyloidosis elsewhere (Jepsen and Nielsen, 1955), just as widespread amyloidosis of the walls of the trachea and bronchi, with or without extension into adjacent aveolar septa, may apparently occur without amyloidosis of other organs and tissues (Noring and Paaby, 1952; Whitwell, 1953). Amyloidosis confined to the lungs also occurs, usually with the formation of tumour-like masses; it has been reviewed recently by Glauser (1955). Amyloid change in the mucosa of the respiratory passages may take the form of small or large plaques, replacing the connective tissue of the mucosa, surrounding and eventually obliterating the mucous glands and spreading outwards between and around the cartilages. In other cases protuberant nodules form in the mucosa; these range from a millimetre or so up to one or two centimetres in diameter. The larger masses may cause bronchial obstruction, with the usual consequences of pulmonary collapse and infection; these masses may closely mimic neoplasms, particularly bronchial adenoma, as seen by bronchoscopy. Widespread amyloid deposition in the alveolar septa may greatly reduce the elasticity of the lungs, so that when the thoracic cavities are opened at necropsy the lungs remain bulky, have a curious glassy pallor, and often feel rather like tough sponge-rubber. Oftener, the involvement of the alveolar septa is patchy, and such lungs may feel finely or coarsely nodular; these deposits account for the miliary or coarser nodular shadowing which may be seen in radiographs.

Some of the amyloid nodules in the lungs, when cut, are gritty, due to calcification, and bone formation may be present. Calcium salts may be laid down either at the centre of the amyloid deposits or at their surface, and calcification is oftener seen in the tumour-like masses of amyloid than in the deposits in the alveolar septa. When bone forms it is usually at the periphery of the larger deposits, between the amyloid and the capsule-like condensation of collagenous tissue which often demarcates it from the adjacent lung structures. Cartilage, osteoid, and true bone may form, and haemopoietic tissue may develop in the medullary spaces of the latter. Glauser (1955) has discussed the occurrence of bone in pulmonary amyloidosis.

When there is widespread amyloidosis of the lungs there is likely to be morbid anatomical

FIG. 14.—Amyloidosis of bronchial wall, extending from cartilage to epithelial lining. Haematoxylin and eosin. $\times 100$.

FIG. 15.—Amyloidosis of alveolar septa in lung. Haematoxylin and eosin. $\times 80$.

FIG. 16.—Heavy interstitial amyloidosis of thyroid. Haematoxylin and eosin. $\times 115$.

FIG. 17.—Amyloidosis of bone marrow: "amyloid rings" round fat cells are clearly seen. Haematoxylin and eosin. $\times 70$.

FIG. 18.—Amyloidosis of haemopoietic bone marrow: in this instance many fat cells appear to have been replaced by amyloid, although the cell outline is preserved, except where the amyloid of adjacent cells has run together. All the homogeneous grey material in this picture is amyloid. Haematoxylin and eosin. $\times 115$.

evidence suggestive of hypertension in the pulmonary circulation, such as pulmonary atherosclerosis and arteriolosclerosis, and hypertrophy of the right ventricle: the occurrence of amyloidosis in the heart and vessels obscures the picture, and makes interpretation of these changes equivocal, although it seems likely that the embarrassed pulmonary circulation in such cases may contribute to the heart failure which is the commonest termination of the disease.

The pleurae may show amyloid involvement comparable to that found in the peritoneum.

Endocrine Glands.—Amyloid deposits in the endocrine glands may be confined to the walls of their larger blood vessels or, in the case of the pituitary and adrenals, to the reticulin fibres along the vascular sinusoids. Functional disturbances are rare. Parenchymatous deposits may occur, and the adrenals may be as severely affected in primary amyloidosis as in any case of secondary amyloidosis. Amyloid goitre (Walker, 1942) is a rare manifestation of primary amyloidosis. The thyroid may be little enlarged, or it may weigh as much as 200 g.; its surface is smooth, unless there has been nodular hyperplasia. The severely affected gland may be remarkably pale and translucent, and its cut surface is without the shiny appearance of thyroid colloid. Microscopically, the acini are widely separated by interstitial deposits of amyloid (Fig. 16).

Skeletal Muscle.—Skeletal muscle may be extensively affected. The diaphragm is said to be particularly liable to involvement. Heavily affected muscles are considerably enlarged by the accumulation of amyloid, which is deposited in connective-tissue spaces and in blood-vessel walls as well as in intimate association with the muscle fibres themselves.

Bones, Joints, and Tendons.—There may be extensive amyloidosis of marrow (Figs. 17 and 18), with replacement of haemopoietic tissue. In long bones amyloidosis is usually severest where haemopoietic tissue is normally present. Occasionally the marrow cavity is more or less filled by amyloid, which may extend throughout the system of Haversian canals: atrophy of bone and pathological fracture may result (Koletsky and Stecher, 1939). In many cases, however, the only amyloid in bones is in blood-vessel walls.

Nodular masses or plaque-like deposits of amyloid may be found in the synovial and capsular tissues of *joints*, and in ligaments, tendon sheaths, retinacula, and bursae. These may cause considerable deformity and interfere with movement,

and are quite similar to the juxta-articular amyloid deposits which are found in some cases of myelomatosis.

Nervous System.—Apart from amyloid deposition in the walls of blood vessels in the meninges and choroid plexuses the tissues of the central nervous system are all but invariably free from involvement in generalized amyloidosis. Amyloid, confined to the central nervous system, may be found within neurones in Alzheimer's disease (Divry, 1934), and as extracellular deposits at the centre of senile plaques (Divry, 1939). Divry (1936, 1941–42) described amyloidosis affecting only the cerebral blood vessels and meninges of old people. These observations are interesting in relation to the problem of the more generalized form of "senile" amyloidosis which Hüsselmann (1955) discussed. Ritama (1954) briefly reviewed the literature relevant to the occurrence of amyloid in the parenchyma of the brain in other circumstances, and reported a case of primary amyloidosis with amyloid deposition in the cerebellar cortex. Ritama put forward the interesting suggestion that altered capillary permeability was an essential prerequisite in the development of parenchymatous amyloidosis of the brain in these cases: according to this view the initial capillary damage, of whatever cause, leads to focal degenerative changes in the brain tissue, which in turn are followed by a proliferative astrocytic reaction, with the eventual deposition of amyloid from the plasma which has permeated the lesion. The postulated alteration in vascular permeability may reflect yet another aspect of the pathological importance of the so-called "blood-brain barrier."

The peripheral nerves, both of the central and autonomic nervous systems, are not infrequently involved in primary amyloidosis, in contrast to the extraordinary infrequency of involvement of the brain and spinal cord. In most cases in which the search has been made some involvement of the blood vessels of nerves has been found. In other cases nodular deposits of amyloid form between the nerve fibres, or in sympathetic and other ganglia, and may lead to complete disorganization of the affected structures. Severe involvement of the peripheral nerves may be the predominant clinical manifestation of primary amyloidosis. Amyloid neuropathy has recently been reviewed by Ritama and af Björkesten (1954).

Skin.—The skin may appear normal in primary amyloidosis; on the other hand its involvement may be the main clinical manifestation. Opaque, opalescent, or translucent nodules or plaques,

xanthoma-like or xanthelasmoid lesions, lichenoid, sclerodermatoid, and eczematized lesions, and purpura may occur. Histologically, amyloid may be found as scattered, minute subepidermal nodules or as extensive deposits throughout the dermis, often involving the subcutaneous tissue, where the typical ringling of the fat cells may be the clue to the diagnosis in biopsy specimens. The epidermis and its appendages are often atrophic in the affected areas. In other cases the amyloid is mainly present in the cutaneous blood vessels; in the absence of associated haematological disorders, cutaneous purpura, which is a fairly common manifestation in primary amyloidosis, is ascribed to haemorrhages from capillaries weakened by amyloid change (Propp, Sharfman, Beebe, and Wright, 1954).

Localized Primary Amyloidosis.—Amyloidosis, apparently confined to a single system or organ, is unusual, but less infrequent than published records might suggest. The situations where localized amyloidosis of this sort have been described include (1) the upper respiratory tract, including the nose, nasal sinuses, and larynx, (2) the lower respiratory tract (trachea and bronchi) and lungs, (3) the urinary bladder and urethra, (4) the skin and orificial mucosae (conjunctiva, mouth, vagina). In such cases the macroscopical and microscopical characteristics do not differ from those presented by involvement of the corresponding parts in cases of generalized amyloidosis. Occasional cases of localized amyloidosis, particularly cutaneous amyloidosis, have been studied at necropsy without evidence of amyloidosis being found in other parts of the body; it is possible that amyloidosis in some cases is in fact strictly localized, although far more comprehensive histological studies of other tissues than have been usual will be needed to substantiate this. Certainly, the chronic course, sometimes with progression of the lesions and sometimes with no apparent change over months or years, indicates that many of these localized cases are more torpid than generalized amyloidosis. Study of such cases over long periods, and meticulously careful and exhaustive post-mortem histological examination, will eventually clarify their natural history and their relation to other forms of amyloidosis. Care is necessary to avoid misdiagnosing hyalinized collagenous nodules, such as are not uncommon in the larynx, as "amyloid tumours": in distinguishing these hyalinized structures from amyloid more weight can be put on their fuchsinophilia when stained with Van Gieson's fluid than on their failure to

FIG. 19.—Appearance suggesting "lacunar resorption" of amyloid by multinucleated giant cells in a lymph node. Haematoxylin and eosin. $\times 180$.

stain metachromatically with rosanilin dyes or their affinity for Congo red.

Cases of senile amyloidosis with the usual predominant involvement of myocardium ought not to be classified as "localized cardiac amyloidosis," as is sometimes done, for in these cases there is always some degree, if sometimes only slight, of amyloidosis elsewhere, usually in blood-vessel walls. The same is true of the occasional cases of what prove histologically to be generalized amyloidosis, although presenting with clinical manifestations or macroscopical changes limited to one organ, such as the stomach, urinary bladder, spleen, or thyroid.

Localized Amyloid Deposits in Tumours.—Very occasionally, material with the typical appearance and staining reactions of amyloid is found in the stroma of tumours, particularly in squamous papillomas of the skin and, reputedly, in some basal-cell carcinomas. The circumstances suggest that this phenomenon is a true local change, unassociated with amyloidosis elsewhere. Its significance is obscure.

Regression of Amyloidosis.—It has long been known that the clinical manifestations of secondary amyloidosis may, rarely, regress completely if

the predisposing disease can be eradicated. By the time it becomes manifest amyloidosis is usually too far advanced for treatment of the predisposing disease to have an effect on the course of the amyloid diseases. Little or nothing is known of the mechanism by which amyloidosis regresses: yet regression can be so complete that gross clinical manifestations, such as the nephrotic syndrome or gross hepatosplenomegaly, may disappear completely. There does not appear to be any record of detailed histological examination of the tissues after regression of amyloidosis: invaluable information might be got from such a study.

Regression of primary amyloidosis has not been reported. The occurrence of a foreign-body giant-cell reaction around the amyloid deposits of some cases suggests a resorptive process (Fig. 19). It is interesting that resorption of experimentally induced amyloidosis in animals has been observed by Dick and Leiter (1941), Richter (1954), and others to occur in association with the local accumulation of macrophages, including multinucleated forms. This does not necessarily mean that amyloid material can only be dispersed by the agency of a cellular reaction.

Aetiology and Pathogenesis

Most of the patients with primary generalized amyloidosis have been in their fifties when the diagnosis was made, although the disease has been observed at all ages. Men are affected twice as commonly as women. No special racial incidence has been observed. A familial incidence has been noted (Andrade, 1952; Kantarjian and DeJong, 1953), and inheritance as a simple Mendelian dominant was reported by Jackson, Rukavina, Block, Falls, and Carey (1955); usually, however, the disease occurs without evidence of any heritable predisposition. The close relationship of the various forms of amyloidosis makes it obligatory to consider them together when discussing the aetiology and pathogenesis of any group of cases of amyloidosis.

Amyloid is of inconstant chemical composition but consists essentially of a protein or complex of proteins usually, if not always, associated with sulphated polysaccharides and, at least on occasion, with lipids (see Hass and Schulz, 1940; Hass, 1942; Hass, Huntington, and Krumdieck, 1943).

Relation to Protein Metabolism.—A relationship between amyloidosis and protein metabolism has long been recognized, particularly since Kuczynski's (1922) experimental production of amyloidosis in mice by feeding casein-rich diets.

An unusually high milk or cheese consumption has been noted in the history of some patients with amyloidosis, e.g., by King and Oppenheimer (1948), but is of doubtful aetiological significance. Jaffé (1926) suggested that primary amyloidosis might result from sensitivity to ingested protein in association with a functional abnormality of the intestine or liver. No convincing evidence has been put forward to indicate that diet or alimentary function plays any part in the occurrence of amyloidosis in man.

Relation to Myelomatosis.—The work of Bayrd and Bennett (1950) and of Snapper, Turner, and Moscovitz (1953) indicates that amyloidosis develops in some 10 to 15% of cases of myelomatosis. The amyloid usually has the distribution seen most frequently in primary amyloidosis, although the picture can be that of classical secondary amyloidosis. It has been suggested that all cases of apparently primary amyloidosis are occult or burnt-out cases of myelomatosis (Apitz, 1940); while this suggestion has been shown not to correspond to the facts it is a reminder of the necessity for considering the possibility of underlying myelomatosis whenever amyloidosis is found. Care must be taken to distinguish between myeloma and simple plasma-cell infiltration, a distinction which can be difficult (Propp *et al.*, 1954; Reimann *et al.*). Material with the staining reactions of amyloid has sometimes been found in the cytoplasm of myeloma cells (Goltz, 1952).

Relation to Hyperglobulinaemia (see Letterer, 1949).—A relationship between plasma-cell proliferation, hyperglobulinaemia, and antibody formation is now well recognized. Hyperglobulinaemia occurs in some cases of amyloidosis. It has been found least regularly in cases of secondary amyloidosis; the reversal of the albumin-globulin ratio which has commonly been noted in secondary amyloidosis seems usually to reflect loss of albumin in the urine and not, as in primary amyloidosis, an absolute increase in the globulin concentration. As hyperglobulinaemia is common in cases of myelomatosis it is interesting that in those cases of myelomatosis in which amyloidosis has developed hyperglobulinaemia is usually absent: moreover, the degree of hyperglobulinaemia in cases of myelomatosis has been observed to fall as amyloidosis develops (Eisen, 1946); on the other hand, nearly all patients with amyloidosis complicating myelomatosis have Bence Jones protein in the urine (Snapper *et al.*, 1953). It is noteworthy that in experimental amyloidosis hyperglobulinaemia seems regularly to develop, and

then declines as amyloid is laid down in the tissues (Dick and Leiter, 1941). These experimental observations suggest the possibility of at least a transitory hyperglobulinaemic phase in human amyloidosis generally. Contrary to what has been maintained by some writers, hyperglobulinaemia is not uncommonly observed in primary amyloidosis.

Few detailed qualitative and quantitative protein studies by modern physicochemical methods have been reported in relation to amyloidosis. Hüsselmann (1955) recorded some such investigations in cases of "senile" amyloidosis. It is clear that studies of this sort, in combination with parallel studies of the amyloid material itself, are likely to advance our knowledge of amyloidosis considerably. There are qualitative differences in the globulin fractions concerned in the hyperglobulinaemia in different cases of amyloidosis, and possibly in individual cases at different times: γ -globulins are generally found to be raised, but may be normal, while there may be a moderate or large increase in the amount of α_2 -globulin and β -globulin.

Relation to Antibody Formation: The Role of Antigen-Antibody Reactions (see Loeschke, 1927; Letterer, 1934).—In those cases of amyloidosis in which infection is a predisposing condition it has been supposed that hyperglobulinaemia results in some way from prolonged production or overproduction of antibodies, and that the excess protein is precipitated in the tissues to form amyloid. The role of chronic infection has alternatively been interpreted as leading ultimately to a failure to produce an adequacy of antibodies, with the consequence that an excess of circulating antigen reacts in the tissues with antibodies already fixed there, the result being the deposition of amyloid, which, according to this hypothesis, is an antigen-antibody complex with which various, and variable, substances (protein, polysaccharide, and lipid) present in the tissue fluids are bound. In many instances the fixed antibody is supposedly concentrated in the so-called reticuloendothelial organs and consequently the amyloid deposits predominate there (see Latvalahti, 1953). Possibly a process of precipitation, once initiated, continues indefinitely so long as fresh antigen continues to enter the circulation and a sufficient supply of antibody with which it can react continues to be available. Again, it is possible that an altered protein by-product of antibody formation is deposited in the tissues, a hypothesis which would help to explain the heavy involvement of certain organs rich in antibody-producing tissue, such as the spleen and, perhaps,

the liver, but which does not account for the involvement, or relative freedom from involvement, of other organs and tissues.

As there is no evidence that infection plays any part in the aetiology of primary amyloidosis, or of amyloidosis complicating myelomatosis, some other basis for an antigen-antibody response would have to be found if this hypothesis were to be maintained for all forms of amyloidosis. It has been suggested that in these cases autosensitization occurs, with the formation of antibodies against the patient's own tissues. Hüsselmann (1955) noted the intimate association of amyloid deposits with cardiac, skeletal, and smooth muscle cells, and suggested that muscle may act as an autoantigen, to which autoantibodies develop, with the eventual occurrence of an antigen-antibody reaction in muscle tissue, morphologically manifested by the deposition of amyloid. Such a mechanism would not readily explain the deposition of amyloid in other tissues, such as adipose tissue, hepatic parenchyma and the glomeruli: an autoantigen of wider distribution than muscle would explain the location of the amyloid more satisfactorily.

Relation to Allergic States.—There is no evidence of a general association between primary amyloidosis and allergic diseases, although in occasional cases there has been an old history of asthma, urticaria, or the like. Pollen allergy had been present in the case of Findley and Adams (1948), and there was a history of allergy to egg protein in the patient of Lindsay (1946). In the case of Baker, De Navasquez, and Maclean (1949) the patient was sensitive to milk, and exclusion of milk and milk products from the diet was associated with some subjective improvement during the course of the illness.

Polysaccharide, Connective-tissue Ground Substances and Amyloidosis.—Faber (1948) noted that amyloid liver has an abnormally high glucosamine content and that the serum glucosamine concentration is raised in amyloidosis and in the presence of suppuration. As glucosamine is a constituent of the mucopolysaccharides, which are present in abundance in the ground substance of healthy connective tissue, he suggested that amyloidosis may represent deposition of excess polysaccharide. Pirani, Bly, and Sutherland (1950) produced amyloidosis by feeding guinea-pigs with a diet deficient in vitamin C, adequate amounts of which are essential for the maintenance of normal ground substance. Such observations as these point to the possibility of a relationship between hyperglucos-

aminaemia, ground substance, and amyloid formation, and suggest a source of the polysaccharide constituent of amyloid. However, the low concentration of polysaccharide in amyloid, not amounting to above 2% (Hass), indicates clearly that other factors than these must have a major role in amyloid formation: investigation of the relationship between the glucosamines and globulins and the development of amyloidosis is called for.

Relation to the So-called Collagen Diseases.—Mallory (1914) and Warren (1930) suggested that amyloid was a product of a perverted activity of fibroblasts in connective tissues generally. Nowadays, such a view necessarily raises the question of the concept of the so-called "collagen diseases" in relation to amyloidosis; Jackson (1954) considered primary amyloidosis to be one of the collagen diseases. A relationship between primary amyloidosis, disseminated lupus erythematosus, and sarcoidosis was postulated by Teilum (1948a, b) on the grounds that they all present the manifestations of "allergic hyperglobulinosis" (hyperglobulinaemia, and "paramyloidosis" proceeding to "hyalinosis" in the reticuloendothelial organs). The idea of a relationship between primary amyloidosis and disseminated lupus erythematosus was further developed by Ritama and Saksela (1949); Ritama and Virkkunen (1953) suggested that a morphological similarity between some of the vascular lesions of primary amyloidosis and the lesions of thrombotic microangiopathy (thrombotic thrombocytopenic purpura) strengthened the case for a relation between primary amyloidosis and disseminated lupus erythematosus, which has a number of points of similarity to thrombotic microangiopathy. However, the supposed morphological similarities described by Teilum and by Ritama and his colleagues are not convincing evidence of any close aetiological or pathogenetic relationship between these various diseases.

Hormonal Factors in Relation to Amyloidosis.—As an aetiological concept, the suggestion that primary amyloidosis is related to the collagen diseases is interesting and stimulating, but one would be unwise to interpret it too readily as a justification for the use of corticotrophin or cortisone in treatment of amyloidosis, as has been suggested by some authors. Peräsalo and Latvalahti (1954) in a thoughtful clinical and experimental study of amyloidosis drew attention to the relationship between amyloidosis and endocrine secretions. They found that corticotrophin and, to a less extent, cortisone encouraged the development of experi-

mental amyloidosis, whereas desoxycorticosterone had no such effect; similarly, castration increased amyloid production while testosterone had no effect; thyroidectomy encouraged amyloidosis, whereas thyroid extract had an inhibitory effect upon its development. Uotila, Peräsalo, and Vapaavuori (1955) found that thyrotrophin accelerated experimental amyloid formation and that growth hormone had no effect.

Three cases bearing on the role of hormonal factors in amyloidosis have been described recently (Case Records of the Massachusetts General Hospital, 1956; Symmers, 1956b). What appears to have been virtually a total thyroidectomy was performed for hyperthyroidism in two of these cases, and in the third the thyroid was evidently destroyed by radio-active iodine: all three patients developed amyloidosis during the following few years, in one case in association with malignant exophthalmos and pretibial myxoedema; in the only surviving case there is evidence of regression of the amyloidosis since treatment with thyroid extract has brought the hypothyroidism under control.

These experimental and clinical observations are of obvious importance and should help to open up a new field of investigation in relation to amyloidosis. Of immediate practical significance is the suggestion, implicit in the Finnish workers' results, that corticotrophin and cortisone may be contraindicated in the treatment of amyloidosis. Paulsen (1950) noted aggravation of the disease in two patients treated with corticotrophin; other workers have recorded no effect, beneficial or otherwise (Milliken, 1955), apart from some improvement after treatment of two cases of localized laryngeal amyloidosis with cortisone and corticotrophin respectively (McCall and Fisher, 1953). Bickel and Lasserre (1956) reported rapid development of amyloidosis during treatment of a case of pulmonary tuberculosis with cortisone.

Therapeutic Agents in Relation to the Developments of Amyloidosis.—The previous paragraph suggests that amyloidosis may prove to be aggravated by certain hormonally active substances. Other drugs may have a similar action. Spain (1956) described the rapid development of amyloidosis in a patient who had been treated with a nitrogen mustard for "Hodgkin's sarcoma," a clinical observation parallel to Teilum's (1954) finding that nitrogen mustard enhanced the experimental production of amyloidosis in mice.

There is no good evidence that drugs can *cause* amyloidosis. Teilum (1948b) recorded a case in which he attributed amyloidosis to previous ad-

ministration of sulphonamide; the relationship is not clear from his report.

Miscellaneous.—Reimann *et al.* (1954) suggested that amyloidosis might be regarded as a form of proteinosis, and they likened it to storage diseases such as glycogenosis (von Gierke's disease) and the lipidoses.

Naturally occurring amyloidosis in animals has been noted, particularly in mice. It is interesting that there is evidence of a hereditary liability to the disease in some strains of mice, and that the incidence in senile mice rises with increasing age (Dunn, 1944). How far natural and experimental amyloidosis in animals can be compared with amyloidosis in man is not yet known.

Clinical Findings in Primary Amyloidosis

Symptomatology.—Primary amyloidosis may present clinically with manifestations due to involvement of any system or organ, but progressive heart failure is by far the most frequent manifestation. The variety of symptoms is almost limitless, reflecting the generalized nature of the disease, and demanding its consideration in the differential diagnosis of many everyday conditions. Manifestations of primary amyloidosis which have been recorded include the following:

Intractable and progressive congestive heart failure (with or without heart block or disturbances in rhythm).

Myocardial infarction (rare).

Dyspnoea (heart failure; pulmonary amyloidosis).

Macroglossia (*not* a constant finding).

Dysarthria (macroglossia).

Dysphagia (macroglossia; oesophageal involvement).

Mikulicz's syndrome and xerostomia (involvement of lachrymal and salivary glands).

Hoarseness (laryngeal involvement).

Changing features (cutaneous and subcutaneous involvement).

Stiffening of fingers (*ditto*).

Muscular stiffness and weakness, possibly associated with severe pain, e.g., lumbago (muscular involvement).

Peripheral neuropathy.

Painful, swollen joints (polyarticular or monoarticular).

Dyspeptic symptoms, with or without pain, which may be of ulcer type (gastric involvement; hepatic involvement).

Pyloric or intestinal obstruction.

Diarrhoea or constipation.

Gastrointestinal haemorrhage (ulceration of amyloid deposits; oesophageal varices, secondary to portal hypertension complicating hepatic amyloidosis; purpura).

Purpura (in about a sixth of cases: athrombocytopenic).

Haematological disturbances (anaemia and leucopenia due to amyloidosis of marrow, malnutrition, etc.; erythrocytosis of unknown pathogenesis; leukaemoid blood pictures).

Asthenia, inanition, cachexia, fever.

Nephrotic syndrome.

Uraemia (prerenal or renal).

Jaundice (hepatic amyloidosis; obstruction of ducts).

Goitre (amyloid goitre, with or without hypothyroidism).

Lymph-node enlargement.

Spontaneous rupture of amyloid spleen.

Spontaneous fracture of amyloid bone.

As would be expected from the wide distribution of the amyloid deposits, these clinical manifestations may occur in bizarre combinations, a circumstance which may be a valuable pointer to the diagnosis.

Evaluation of the symptoms and signs may be difficult because involvement of different systems so often overlaps: cardiac, broncho-pulmonary, renal, hepatic and gastrointestinal involvement may contribute to the development of such manifestations as dyspnoea, oedema, proteinuria, uraemia, anorexia, vomiting, constipation, diarrhoea, hepatosplenomegaly, and so on.

Cardiological and Radiological Studies

Electrocardiographic investigations characteristically show low voltage in all leads, and there may be lowering of the S-T segments or inversion of T-waves, delayed conduction and disordered rhythm. Cardiac catheterization studies (Hetzel, Wood, and Burchell, 1953; Gunnar, Dillon, Wallyn, and Elisberg, 1955) have confirmed the clinical impression of earlier observers that the cardiological picture of amyloidosis strikingly resembles that of constrictive pericarditis in its manifestations, course, and lack of response to treatment (Findley and Adams, 1948; Couter and Reichert, 1950). This resemblance may be attributed to the inelasticity of the amyloid myocardium interfering with diastolic expansion of the heart (Gunnar *et al.*, 1955).

The *radiological findings* in amyloidosis of various organs have recently been described by Wang and Robbins (1956).

Diagnostic Investigations

Congo Red Test.—The intravenous Congo red test is less regularly positive in primary amyloidosis than in secondary amyloidosis, a finding which reflects the higher proportion of primary cases in which the amyloid has little or no affinity for the dye. While a negative result does not rule

out the possibility of amyloidosis, opinions differ on the degree of retention of the dye which may be considered to indicate a positive result. Stemmerman and Auerbach (1944) advised that 90% retention was necessary for diagnosis; this figure is arbitrary, and judgment must be used in interpreting lower results. Care must be taken to ensure, as far as possible, that the intravenous Congo red test is not done in cases in which superficially situated amyloid deposits are likely to be unsightly if stained by the dye.

In some cases superficial lesions may be stained by injecting a small amount of Congo red solution in their immediate vicinity: at least 48 hours should be allowed to pass before reading the result of this local dye test, in order that there may be time for dispersal of dye which is not bound to amyloid.

Other Chemical Investigations.—Measurement of the serum proteins and protein-bound polysaccharides and their study by electrophoresis on paper can give information which is of value in the diagnosis of amyloidosis (Gilliland and Stanton, 1954). Gilliland and Stanton (1954) found that estimation of the albumin/ α_2 -globulin ratio derived from simple paper electrophoresis of the serum proteins was useful in studying amyloidosis and some other conditions; an albumin/ α_2 ratio below 3 was of diagnostic help in amyloid disease.

Biopsy.—Biopsy is often a very helpful method of investigation, but does not always give positive results in cases which ultimately prove to be primary amyloidosis. Its value is greatest when a definite clinical lesion is accessible, such as macroglossia, enlarged skeletal muscles, juxta-articular nodules, skin lesions, and the like: "blind biopsy" of clinically normal tissues is often unrewarding.

Gum biopsy has become a popular procedure, but is less often helpful in primary amyloidosis than in secondary amyloidosis; Selikoff and Robitzek (1947) found amyloid in the gum in 14 out of 47 cases of amyloidosis complicating tuberculosis, whereas a considerably smaller proportion of positive results has been recorded in the literature of primary amyloidosis. As a practical point it is worth commenting that biopsy specimens of gum are usually small, and the fixity of the tissue commonly results in considerable surgical trauma to the specimens, making them quite unsatisfactory for histological study.

Tongue biopsy may give negative results, even in the presence of gross amyloid macroglossia: this is generally due to too small and superficial a specimen being taken, for all too often only

lingual mucosa is included in the specimen, and the mucosa is not always involved. The deeper incision necessary to obtain a representative piece of lingual muscle may not heal readily if the tongue is severely affected by amyloidosis, and the operation and post-operative period are often most distressing to the patient.

Needle biopsy of liver, spleen, or kidney can prove valuable. Needling liver or spleen may carry the hazard of haemorrhage, particularly if the organ is unusually inelastic because of the presence of much amyloid. Fatal haemorrhage complicated needle biopsy of the liver in the case of Volwiler and Jones (1947); others have performed many needle biopsies of amyloid livers without serious complications resulting (Waldenström, 1928). Some workers consider formal excision of a piece of tissue at open operation to be preferable to puncture. Serial needle biopsies of kidney were studied by Muehrcke, Pirani, Pollack, and Kark (1955) in a case of primary amyloidosis with the nephrotic syndrome.

Lymph-node biopsy is an obvious procedure in cases presenting with lymphadenopathy.

Sternal marrow aspirates have been shown to contain gelatinous masses of what appeared to be amyloid (Propp, *et al.*, 1954); the interpretation of such appearances could be uncertain in the absence of specific staining characteristics. Rib biopsy might be valuable, but there is as yet little information about the frequency of amyloid deposits in rib marrow.

In interpreting biopsies care must be taken to avoid mistaking hyalinized collagen for amyloid; the value of Van Gieson's fluid as a differential stain has already been mentioned. Apart from hyalinized collagen there are few conditions which need to be noted in the histological differential diagnosis. As Ritama and Virkkunen (1953) pointed out, there is some resemblance to the vascular lesions of thrombotic microangiopathy, but the clinical picture of the latter and the absence of extravascular lesions make confusion unlikely (Symmers, 1956b). The lesions of giant-cell arteritis (Harrison, 1948) have some similarity to the juxta-vascular form of amyloidosis with giant-cell reaction, but again confusion between the two conditions is unlikely.

Laboratory Findings in Primary Amyloidosis

The urine may be normal, but protein is usually present and is likely to be abundant if there is parenchymatous renal involvement. Amyloid casts are rarely found. Hypoproteinaemia, due wholly or partly to loss of protein in the urine,

may occur; hyperglobulinaemia is commoner. Hypercholesterolaemia is usually associated with the nephrotic syndrome but is occasionally observed in the absence of any evident renal damage. The basal metabolic rate may be below the usual range. High serum levels of alkaline phosphatase have been reported in association with severe skeletal and hepatic amyloidosis (Wolf, Hitzig, and Otani, 1955). Other chemical investigations give results according with the visceral involvement and the resulting functional disturbances.

The erythrocyte sedimentation rate is usually considerably raised. Haematological examination may show little or no abnormality. In some cases progressive hypochromic anaemia develops; occasionally, erythrocytosis has been observed in the absence of any evident predisposing circumstance (Husten, 1924; Wolf *et al.*, 1955). Leucopenia may be present; sometimes an absolute lymphocytosis or monocytosis occurs. Purpura has not been associated with thrombocytopenia or with any detected disturbance in the clotting mechanism, and is generally attributed to fragility of vessels severely affected by amyloid infiltration (Propp *et al.*, 1954).

Prognosis and Treatment of Primary Amyloidosis

Neither spontaneous nor therapeutically induced recovery has been observed in primary amyloidosis. Most reported cases have ended fatally within one to three years of the first symptoms; shorter survivals and survival for several years have been recorded.

No effective treatment is known. Haemmerli (1954) suggested that treatment with thyroid extract might be tried in cases of primary amyloidosis, his grounds being that hypothyroidism consequent upon amyloid involvement of the thyroid may contribute to the clinical picture. Experimental evidence also suggests that thyroid extract deserves trial (Peräsalo and Latvalahti, 1954). The presence in many cases of serious myocardial embarrassment indicates the caution needed in giving such treatment. As already mentioned, there are conflicting views on the value of corticotrophin and cortisone in therapy of this disease; experimental evidence indicates that they may be contraindicated, and practically all therapeutic trials have been entirely without benefit.

Spain (1956) drew attention to the possible risk of enhancing amyloid formation by treating predisposing diseases with nitrogen mustard in the presence of acute or chronic suppuration. This indicates that the use of nitrogen mustards as

empirical treatment for primary amyloidosis, as has been advocated, although apparently not practised, may in fact be contraindicated.

When primary amyloidosis comes to be considered regularly in differential diagnosis its accurate earlier diagnosis will enable thorough studies to be made, with a view to elucidating the aetiological problems in general and to allowing trials of therapeutic measures, which clearly should include assessment of the effects of hormones.

Summary and Conclusions

In sum, the aetiological and pathological and clinical differences between generalized amyloidosis occurring without a recognizable predisposing cause ("primary amyloidosis") and generalized amyloidosis complicating some other disease ("secondary amyloidosis") are not greater than the differences between individual cases within either category. It is true that in the majority of cases of secondary amyloidosis, excluding amyloidosis complicating myelomatosis, certain viscera are predominantly affected, and this is reflected in the clinical manifestations. It is equally true that other tissues are predominantly affected in the majority of cases of primary amyloidosis, and of amyloidosis complicating myelomatosis, and consequently a different group of clinical manifestations is characteristic of these cases. In *all* types of generalized amyloidosis, however, the same tissues are affected: the difference lies in the relative preponderance of the amyloid change in this group of organs or that.

If it were the rule for every case of amyloidosis occurring as a complication of other diseases to conform to one certain pattern, and for every case of primary amyloidosis to conform to a different pattern, it would be justifiable to define a series of "amyloid diseases." The fact that, instead of such constant patterns, any manifestation of amyloidosis may occur in association with any obvious predisposing disease, or with none, suggests that all varieties of amyloidosis are related manifestations of one fundamental disturbance. The nature of the fundamental disturbance is quite obscure, although something is beginning to be known of possible pathogenetic mechanisms which may be concerned.

The view which is now most generally held is that amyloidosis is a manifestation of an immunological disturbance, with the deposition in prepared situations of amyloid, a substance of somewhat varied composition which may be the insoluble glycoprotein product of a local antigen-antibody reaction. It is becoming apparent that

polysaccharides and connective-tissue ground substance as well as serum globulins have a role in the pathogenesis of amyloidosis; the influence of hormones is now also attracting serious attention. Whatever the mechanism of amyloid formation may be, it seems probable that the factors leading up to its deposition may vary; the predisposing cause, when known, is clearly only one of a number of factors concerned. The bias towards a different predominant location of the deposits in primary and secondary amyloidosis may reflect a different balance in the interaction of a number of interrelated pathogenetic mechanisms, and this difference may be determined by different types of stimulus operating to put these mechanisms into action.

It is a pleasure to express my indebtedness to Mr. Brian Armitage, Librarian to Charing Cross Hospital Medical School, for his invaluable help in collecting the vast number of publications relating to amyloidosis.

I wish to thank Mr. K. W. Iles, of the School's photographic department, for his help in preparing the photographs.

REFERENCES

- Andrade, C. (1952). *Brain*, **75**, 408.
 Apitz, K. (1940). *Virchows Arch. path. Anat.*, **306**, 631.
 Baker, G. P., De Navasquez, S., and MacLean, K. S. (1949). *Guy's Hosp. Rep.*, **98**, 95.
 Bayrd, E. D., and Bennett, W. A. (1950). *Med. Clin. N. Amer.*, **34**, 1151.
 Beames, R. P. (1955). *J. Urol. (Baltimore)*, **73**, 804.
 Bickel, G., and Lasserre, R. (1956). *Bull. Soc. méd. Hôp. Paris*, **72**, 275.
 Budd, G. (1845). *On Diseases of the Liver*, ch. 3, Sect. 3. Churchill, London.
 — (1857). *Ibid.*, 3rd ed., ch. 3, Sect. 3. Churchill, London.
 Case Records of the Massachusetts General Hospital (1956). *New Engl. J. Med.*, **254**, 226.
 Cooley, R. N. (1953). *Amer. J. Roentgenol.*, **70**, 428.
 Couter, W. T., and Reichert, R. E., Jr. (1950). *Circulation (N.Y.)*, **2**, 441.
 Dahlin, D. C. (1949). *Ann. intern. Med.*, **31**, 105.
 — (1950). *Med. Clin. N. Amer.*, **34**, 1107.
 Dick, G. F., and Leiter, L. (1941). *Amer. J. Path.*, **17**, 741.
 Divry, P. (1934). *J. belge Neurol. Psychiat.*, **34**, 197.
 — (1936). *Ibid.*, **36**, 24.
 — (1939). *Ibid.*, **39**, 444.
 — (1941–42). *Ibid.*, **41**, 141.
 Drapiewski, J. F., Sternlieb, S. B., and Jones, R. (1955). *Ann. intern. Med.*, **43**, 406.
 Dunn, T. B. (1944). *J. nat. Cancer Inst.*, **5**, 17.
 Eisen, H. N. (1946). *Amer. J. Med.*, **1**, 144.
 Faber, M. (1948). *Acta med. scand.*, Suppl. 206, p. 351.
 Findley, J. W., Jr., and Adams, W. (1948). *Arch. intern. Med.*, **81**, 342.
 Gilliland, I. C., and Stanton, E. (1954). *Journal of Clinical Pathology*, **7**, 172.
 Glauser, O. (1955). *Schweiz. Z. Path. Bakt.*, **18**, 42.
 Goltz, R. W. (1952). *Medicine (Baltimore)*, **31**, 381.
 Gunnar, R. M., Dillon, R. F., Wallyn, R. J., and Elisberg, E. I. (1955). *Circulation (N.Y.)*, **12**, 827.
 Haemmerli, A. (1954). *Schweiz. med. Wschr.*, **84**, 1262.
 Harrison, C. V. (1948). *Journal of Clinical Pathology*, **1**, 197.
 Hass, G. M. (1942). *Arch. Path. (Chicago)*, **34**, 92.
 — Huntington, R., and Krumdieck, N. (1943). *Ibid.*, **35**, 226.
 — and Schulz, R. Z. (1940). *Ibid.*, **30**, 240.
 Hetzel, P. S., Wood, E. H., and Burchell, H. B. (1953). *Proc. Mayo Clin.*, **28**, 107.
 Higbee, D. R., and Millett, W. D. (1956). *J. Urol. (Baltimore)*, **75**, 424.
 Higgins, W. H., and Higgins, W. H., Jr. (1950). *Amer. J. med. Sci.*, **220**, 610.
 Hüsselmann, H. (1955). *Virchows Arch. path. Anat.*, **327**, 607.
 Husten, K. (1924). *Ibid.*, **248**, 450.
 Jackson, A. (1954). *Arch. intern. Med.*, **93**, 494.
 Jackson, C. E., Rukavina, J. G., Block, W. D., Falls, H. F., and Carey, J. (1955). *J. Lab. clin. Med.*, **46**, 925.
 Jaffé, R. H. (1926). *Arch. Path. Lab. Med.*, **1**, 25.
 Jepsen, O., and Nielsen, K. (1955). *Acta med. scand.*, **151**, 321.
 Kantarjian, A. D., and DeJong, R. N. (1953). *Neurology*, **3**, 399.
 King, F. H., and Oppenheimer, G. D. (1948). *Ann. intern. Med.*, **29**, 374.
 King, L. S. (1948). *Amer. J. Path.*, **24**, 1095.
 Koletsky, S., and Stecher, R. M. (1939). *Arch. Path. (Chicago)*, **27**, 267.
 Kuczynski, M. H. (1922). *Virchows Arch. path. Anat.*, **239**, 185.
 Larsen, R. M. (1930). *Amer. J. Path.*, **6**, 147.
 Latvalahti, J. (1953). *Acta endocr. (Kbh.)*, Suppl. 16.
 Letterer, E. (1934). *Virchows Arch. path. Anat.*, **293**, 34.
 — (1949). *J. Path. Bact.*, **61**, 496.
 Lindsay, S. (1946). *Amer. Heart J.*, **32**, 419.
 Loeschke, H. (1927). *Beitr. path. Anat.*, **77**, 231.
 Lubarsch, O. (1929). *Virchows Arch. path. Anat.*, **271**, 867.
 McCall, J. W., and Fisher, W. R. (1953). *Ann. Otol. (St. Louis)*, **62**, 316.
 McDonald, J. H., and Heckel, N. J. (1956). *J. Urol. (Baltimore)*, **75**, 122.
 Mallory, F. B. (1914). *The Principles of Pathologic Histology*, p. 103. Saunders, Philadelphia and London.
 Mathews, W. H. (1954). *Amer. J. med. Sci.*, **228**, 317.
 Milliken, J. A. (1955). *Canad. med. Ass. J.*, **73**, 458.
 Muehrcke, R. C., Pirani, C. L., Pollack, V. E., and Kark, R. M. (1955). *Guy's Hosp. Rep.*, **104**, 295.
 Noring, O., and Paaby, H. (1952). *Acta path. microbiol. scand.*, **31**, 470.
 Paulsen, F. (1950). *Acta endocr. (Kbh.)*, **5**, 292.
 Peräsalo, O., and Latvalahti, J. (1954). *Acta path. microbiol. scand.*, **34**, 208.
 Peters, J. T. (1943). *Arch. Path. (Chicago)*, **35**, 832.
 Pirani, C. L., Bly, C. G., and Sutherland, K. (1950). *Ibid.*, **49**, 710.
 Pocock, D. S., and Dickens, J. (1953). *New Engl. J. Med.*, **248**, 359.
 Proceedings of the Physiological Society of Edinburgh (1854). *Monthly J. med. Sci.*, **18**, 185.
 Propp, S., Scharfman, W. B., Beebe, R. T., and Wright, A. W. (1954). *Blood*, **3**, 397.
 Randall, O. S. (1933). *Amer. J. Cancer*, **19**, 838.
 Ranström, S. (1951). *Acta path. microbiol. scand.*, **28**, 366.
 Reimann, H. A., Koucky, R. F., and Eklund, C. M. (1935). *Amer. J. Path.*, **11**, 977.
 — Sahyoun, P. F., and Chaglassian, H. T. (1954). *Arch. intern. Med.*, **93**, 673.
 Richter, G. W. (1954). *Amer. J. Path.*, **30**, 239.
 Ritama, V. (1954). *Ann. med. intern. Fenn.*, **43**, 51.
 — and Björkstén, G. af (1954). *Ibid.*, **43**, 152.
 — and Saksela, N. (1949). *Ibid.*, **38**, 188.
 — and Virkkunen, M. (1953). *Ibid.*, **42**, 149.
 Rokitsky, C. (1842). *Handbuch der pathologischen Anatomie*, vol. 3, pp. 311, 384, 424. Braumüller and Seidel, Vienna.
 Schmidt, M. B. (1904). *Verh. dtsch. path. Ges.*, **7**, 2.
 Selikoff, I. J., and Robitzek, E. H. (1947). *Amer. J. Path.*, **23**, 1099.
 Shnider, B. I., and Burka, P. (1955). *Gastroenterology*, **28**, 424.
 Snapper, I., Turner, L. B., and Moscovitz, H. L. (1953). *Multiple Myeloma*, pp. 85–94. Grune and Stratton, New York.
 Soyka, J. (1876). *Prag. med. Wschr.*, **1**, 165.
 Spain, D. M. (1956). *Amer. J. clin. Path.*, **26**, 52.
 Stemmerman, M. G., and Auerbach, O. (1944). *Amer. J. med. Sci.*, **208**, 305.
 Strauss, A. (1933). *Virchows Arch. path. Anat.*, **291**, 219.
 Symmers, W. St. C. (1956a). *Lancet*, **1**, 592.
 — (1956b). *Journal of Clinical Pathology*, **9**, 212.
 Teilum, G. (1948a). *Amer. J. Path.*, **24**, 389.
 — (1948b). *Ibid.*, **24**, 409.
 — (1954). *J. Lab. clin. Med.*, **43**, 367.
 Uotila, U., Peräsalo, O., and Vapaavuori, M. (1955). *Acta path. microbiol. scand.*, **37**, 322.
 Virchow, R. (1854a). *Virchows Arch. path. Anat.*, **6**, 135.
 — (1854b). *Ibid.*, **6**, 268.
 — (1854c). *Ibid.*, **6**, 416.
 — (1855a). *Ibid.*, **8**, 140.
 — (1855b). *Ibid.*, **8**, 364.
 Volwiler, W., and Jones, C. M. (1947). *New Engl. J. Med.*, **237**, 651.
 Waldenström, H. (1928). *Acta chir. scand.*, **63**, 479.
 Walker, G. A. (1942). *Surg. Gynec. Obstet.*, **75**, 374.
 Wang, C. C., and Robbins, L. R. (1956). *Radiology*, **66**, 489.
 Warren, S. (1930). *Amer. J. Path.*, **6**, 161.
 Whitwell, F. (1953). *Thorax*, **8**, 309.
 Wild, C. (1886). *Beitr. path. Anat.*, **1**, 175.
 Wilks, S. (1856). *Guy's Hosp. Rep.*, (Series 3), **2**, 103.
 Wolf, R. L., Hitzig, W. M., and Otani, S. (1955). *Arch. intern. Med.*, **95**, 141.
 Wollaefer, E. E. (1950). *Med. Clin. N. Amer.*, **34**, 1113.