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Gerontologie

Senile Amyloidosis in Mice

By T. J. Thung

the Antoni van Leeuwenhoekhuis, the Netherlands Cancer Institute, Amsterdam.
Gerontological Division, Head Dr.O.Mühlbock.

SENILE AMYLOIDOSIS IN MICE

and the relation between amyloid and ageing in comparative pathology. [1)]

By P.J. Thung.

1. Introduction.

In human pathology, various incidental observations have been made on the occurence of senile tissue changes comparable to amyloid infiltration. King (1948) described 5 cases of atypical amyloid deposits, mainly in the heart, which had in common the absence of causative disease and the advanced age of the subjects. In a large series of autopsies Hüsselmann (1955) reported a 10-15 % incidence of cardiac amyloidosis without known cause in persons over 70 years old. Senile amyloidosis was also reported in the pancreas (Gellerstedt, 1938) and in the seminal vesicles (Bursell, 1941), while Divry (1927) demonstrated the amyloid nature of senile plaques in the brain.

In ageing mice, amyloid disease without known cause has been described as a frequent occurrence in some inbred strains (Dunn, 1944). When working with large colonies of inbred mice which are allowed their natural life span, one is struck by the preponderance of amyloid infiltration and its sequelae among the senile degenerative conditions and the causes of death in certain strains (Mühlbock, 1956).

The following data may serve to illustrate the nature and frequency of these lesions in untreated mice of various inbred

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strains. Our findings in mice and comparable conditions in other species and in man are discussed in relation with the problem of amyloidosis.

2. Material and methods.

The observations recorded in this report were made on untreated mice kept in our laboratory as control animals in various oncologic experiments. Unless otherwise stated the animals were kept until they either died or had to be killed as, by outward signs, death seemed to be imminent. The nomenclature used for inbred strains is according to the Committee on standardized Nomenclature for inbred Strains of Mice (1952). The animals were kept in glass cages (17 x 11 x 12 cm), 4 per cage, and fed commercial pellets and twice a week a handful of wheat. Tap water was provided ad libitum. Tissues preserved for histological purposes were fixed in Susa solution and routine sections were stained with haematoxylin-azophloxin. In addition, other fixations and stains were used as described later on (§ 3).

3. The nature of protein infiltrations in senile mouse tissues.

In routine sections of tissues from old mice, one often finds deposits of an apparently homogenous, slightly eosinophilic substance which infiltrates and surrounds the walls of small blood vessels, occupies interstitial spaces, and in compact parenchymal tissues encroaches upon cellular boundaries and replaces the cytoplasm. While its relation to capillary and other small vessels is constant, its further distribution in the tissues depends upon the architecture of the organ concerned. In loosely build interstices, as in the renal papilla, the reticular zône of the adrenals or the stroma of intestinal villi, it first fills the spaces between collagenous and reticular fibres against which it becomes apposed. Atrophy of adjacent cells is clearly secondary (Figs.2, 10, 13, 21). In other sites, however, the localisation may appear to be primarily epi- or intracellular. This is seen a.o. in fat tissue, in the corpora lutea of the ovaries, and in some cases of adrenal cortical involvement. Here too, such deposits originate around small vessels, but the close relation between vascular wall and cellular boundary causes early invasion of the cytoplasm.(Figs.7,11)

Often the infiltration is grossly discernible: grayish glassy masses pervading the splenic pulp, or yellow-whitish opacity of the normally light red and translucent renal papilla.

This substance suggests amyloid, if only because no other term is better fitting. There are, however, additional reasons for calling this material amyloid, the first of which is that it has been diagnosed as such since 1897. For within the limits of optical methods, the infiltrations described above are identical to what is found in experimentally induced amyloidosis in mice.

Since Davidsohn (1897), injecting bacterial suspensions into various animals, found a higher incidence of amyloid in mice than in other mammals, these animals have been favourite objects in amyloid research. Many descriptions have been given of experimentally induced amyloid in mouse organs, which closely parallel our observations in untreated old mice. The relation between both conditions is discussed in § 6, but mention should now be made of the staining reactions which are often held to be decisive in diagnosing amyloid. These reactions too tend to confirm the identity of experimental amyloidosis in mice and the senile infiltrations described here. Most authors on experimental amyloidosis report positive Congo red and metachromatic gentian-violet or methyl-violet stains. With toluidine blue Teilum (1952) found no metachromasia. Iodine and iodine-sulfuric acid stains are often stated to be positive, though this implies color shades varying from brown to rosa for the iodine stain and from brown to greenish blue for iodine-sulfuric acid (Davidsohn 1897, Kuczynski 1922, Jaffé 1926, Turnbull 1945). There is some variation in the findings of different authors on experimental amyloidosis in mice, much of which may be accounted for by differences in fixation and staining technique. Twort and Twort (1932) describe changes in the spleen, the adrenals and other organs of mice painted with tars and oils, which are identical to experimentally induced amyloidosis. Because positive amyloid staining reactions were not obtained, these authors used the term hyaline degeneration. They failed, however, to indicate the stains and fixatives used. Turnbull (1945), investigating the nature of similar incidental findings in mice treated with pentose nucleotides, found that after alcohol fixation positive iodine and iodine-sulfuric acid stains were obtainable, while formaline fixation prevented this reaction. In fact, Jaffé (1926)

already stressed the necessity of alcohol fixation for iodine staining reactions on experimental mouse amyloid.

Similarly in spontaneous amyloidosis in ageing mice, differences in technique may confuse the findings. Gorer (1940), describing the spontaneous glomerular lesions which were later diagnosed as amyloid by Dunn (1944), found negative iodine, methyl-violet and gentian-violet reactions and therefore speaks of hyaline degeneration. His fixation, however, is not specified. Dunn (1944), using formaline fixed frozen sections, found positive Congo red staining, dubious metachromasia with methyl-violet, and only exceptionally a positive iodine-sulfuric acid stain. Our own findings on the material found in untreated old mice may be summarised as follows.

The material, which in routine sections stains a light rosa with eosin or azophloxin, takes connective tissue stains in Mallory, Masson trichrome, or van Gieson preparations. It always stands out against collagen, however, by assuming a much lighter shade. With Schiffs periodic acid-leucofuchsin treatment a weak positive reaction is obtained in paraffin sections after Susa fixation. Iodine staining with dilute Lugol solution (J_2 : 1, KJ : 2, H_2O : 300) did not differentiate the material from the surrounding tissue in paraffin sections after Susa fixation. In frozen sections after alcohol 85 %, however, a dark brown color was seen though this faded away soon. In frozen sections after alcohol 85 % and to a lesser degree after formalin 4 % fixation, a rosa or brownish violet color was seen when, after treatment with highly dilute Lugol solution, 1 % sulfuric acid was added. This reaction too was highly transitory. Gentian-violet gave no metachromatism in paraffin sections after Susa, formalin or alcohol fixation, though the material assumed a somewhat intenser violet hue

than the surrounding tissues. In frozen sections after formalin fixation the material similarly stood out against the background without showing true metachromatism, as was also reported by Dunn (1944). In frozen sections after alcohol fixation, however, a bright rosa metachromatism was seen which, though fading somewhat, was well preservable. With toluidine blue no metachromatism was obtained in paraffin sections, ~~after alcohol or formalin fixation,~~ but frozen sections after alcohol fixation again gave a clear metachromatic reaction. The substance readily stained with Congo red in paraffin sections after alcohol or formalin fixation. After Susa fixation this reaction was much impaired. Intravital staining with 1 % Congo red in saline, 2 subcutaneous doses of 1 ml. each being given within 48 hours before death, gave good results in frozen sections as well as after paraffin embedding, using a haematoxylin counterstain. For the paraffin sections a concentrated sublimate fixation was used, but formalin will probably do as well. Congo red markedly increases the birefringence of the material, which in unstained preparations is slight. Other treatment, especially the Mallory stain, may impair or even destroy this property.

Do these results entitle us to the use of the term amyloid? This question has also been faced by early workers on experimental amyloidosis who were especially embarrassed by their failure to obtain a true blue iodine-sulfuric acid reaction. It was argued (Davidsohn 1897) that in human amyloid too, iodine stains often failed to give typical reactions. In such cases the combination of optical appearance and a metachromatic reaction with one of the anilin dyes warranted the diagnosis amyloid even if the iodine-sulfuric acid method yielded a reddish brown color only. After the introduction, in 1922, of

the Congo red stain for amyloid, interest in the iodine methods gradually declined and it is noteworthy that in a fairly recent general survey on laboratory and biopsy diagnosis of amyloid, iodine stains are not even mentioned (Dahlin c.s. 1950). The Congo red method itself too has devaluated through the years: Letterer in 1926 regarded it as the best amyloid test, while Dahlin c.s.(1950) point out that a negative Congo red stain does not rule out amyloid. In general, the trend has been to attach a decreasing importance to all specific staining reactions because it was increasingly appreciated that conditions which clinically and pathologically have to be grouped under the general heading amyloidosis, may involve highly variable chemical substances. Formerly this variability, both in experimental and in human amyloid, was thought to correspond to various stages in a process leading to one final end substance (Davidsohn 1897, Schmidt 1904). The establishment as a distinct pathological concept, of atypical forms of amyloidosis, distinguished i.a. by highly variable staining reactions (Lubarsch 1929), implied the notion of several varieties of amyloid substance. Chemical investigations later confirmed that differences in composition are not related to differences in developmental stage or age of the amyloid substance (Hass e.a. 1943). The chemical variability rather reflects the fact that chemical methods are more discriminating than optical or even tinctorial observations. The more refined the chemical analysis, the more complex the problem of the identy of amyloid will become. Hass e.a. (1943), determining solubility ranges of amyloid from various human and animal sources, still found general agreement between pathogenetically comparable cases within a given species. Recent chromatographic studies on amino-acid content (Letterer e.a. 1955) or paper electrophoresis of protein and mucoprotein

fractions (Wagner 1955), however, reveal striking differences even between similar cases of human amyloid.

Thus it is increasingly realised that the term amyloid comprises a wide variety of substances, corresponding perhaps to a similar variety of pathological processes. At the same time, however, this implies that, as long as a more discriminating nomenclature has not been established, histological criteria should carry more weight in deciding the diagnosis, than tinctorial niceties. For the condition observed in mice, the only alternative to amyloid at present is hyaline degeneration. This in fact was the diagnosis chosen by Twort and Twort (1932) and by Gorer (1939). This choice, however, disregards the fact that histologically these infiltrations do not resemble any form of hyaline degeneration known in human pathology, while they are highly similar to amyloidosis. That, moreover, with certain technical specifications, tinctorial similarity is also demonstrable, is a secondary reason only for speaking of mouse amyloidosis.

4. Histological characteristics of amyloid infiltration in senile mice.

The general characteristics of amyloid infiltrations in senile mice are similar to what is known from human pathology. This concerns the relation to vascular walls and perivascular spaces, the accretion to or infiltration between collagenous and reticular fibres, and the secondary damage to parenchymal cells. Yet histologically the lesions in mice differ in various respects from comparable conditions in man, as will be apparent from the following descriptions.

I. Kidneys. Among the organs affected by senile amyloidosis, the kidneys often are most conspicuous because of secondary changes brought about by amyloid deposits in the renal papilla as reported by Dunn (1944 and 1949). Amyloid may appear in the mouse kidney at 3 sites: the glomeruli, the interstitium of cortex and medulla, and the interstitium of the distal part of the renal papilla. The latter site is most common and leads to the grossly discernible lesions for which Dunn (1944) proposed the name papillonephritis. In short the pathogenesis of these lesions is as follows. Amyloid is deposited in the interstitial spaces between the papillary ducts and leads to functional impairment of the orifices of the terminal excretory canals. It also disturbs, of course, the capillary vessels and the Henle loops in this region. Signs of obstruction of tubular flow ensue, viz. dilatation and atrophy of the tubules and dilatation of glomerular capsules. Perivascular infiltrates of lymphocytes and plasma cells are a frequent finding. In some cases frank pyelonephritis with leucocytic infiltration may be apparent, but this occurs in late stages only and clearly is of a secondary nature. The papillary tip meanwhile undergoes

complete necrosis. Atrophy and desquamation of the epithelia leaves an amyloid skeleton of the original structure which becomes washed out, loses its stainability and is finally sloughed off. Longitudinal sections of the papilla at various stages of this process are shown in the papers of Gorer (1939) and Dunn (1944). In addition our figs 1-4 may illustrate the nature of these changes. Parenchymal atrophy in the cortex usually starts in wedge-like areas, but finally involves the entire kidney. By then the kidneys are small and pale and studded with small cysts, originating from glomerular capsules. There usually is little fibrous reaction: the "scarred" areas consist of just a sponge-like network of reticular fibres between which the tubular cells have atrophied. The glomerular capsule-cysts show considerable variation in size and may lead to definitely cystic kidneys, to which tubular dilatation also contributes. The tendency to cystic deformation appears to be genetically determined, as in some strains the small atrophic kidney is the usual final condition, while in others large cystic kidneys are frequent. In both conditions amyloidosis of the papilla as described above is invariably found. Its role in the pathogenesis of these lesions is moreover demonstrated by the fact that this amyloid infiltration is first observed in otherwise as yet completely normal kidneys, while the cortical changes gradually develop as necrosis of the papilla progresses. For this process, to which ascending infection may add a secondary pyelonephritic aspect, papillonephritis is a very apt expression.

Besides the papillary deposits, amyloid is often found in the glomeruli, while sometimes the cortical interstitium between the tubules may also be affected. As stated by Dunn (1949), these 3 sites may be infiltrated either together or independently. It is our experience, however, that in all cases where the

glomeruli are affected, the papilla too will show some degree of amyloidosis. On the other hand, severe papillonephritis may occur without glomerular involvement. This is what happens in strain A mice as found by Gorer (1939), Dunn (1944) and ourselves. Some data on the relative importance of the 3 sites of renal amyloidosis are given in ~~table 3~~ figure 23. The condition is always bilateral, though differences in the degree of damage to left and right kidney are often seen. In these differences no preference for either side was found. In a series of 108 papillonephritic mice, for instance, we found the left kidney most seriously damaged in 32 cases, the right kidney in 25 cases, while in the remaining 51 cases both sides were equally affected. Sex differences were not observed, the incidence of gross papillonephritis being about equal in males and females of the same strain at the same age level.

Finally it should be noted that practically all renal amyloid is related to capillary vessels only. In the comparatively rare cases of interstitial infiltration of the cortex or proximal medulla small veins may be involved, but the arteries are completely intact, and so are the afferent arterioles to amyloidotic glomeruli. ~~(fig. .....)~~

II. Adrenals. In the adrenals too, amyloid is related to pericapillary spaces. It is found especially in the perimedullary zone where a band of vascular reticular tissue separates the cortical and medullary parts of the mouse adrenal. Amyloid is first found as an accretion to the reticular fibres just outside the capillary endothelium. Later these vessels are surrounded and compressed by thick bands of amyloid, and atrophy of all cellular elements in this zone ensues. Finally amyloid may spread into the peripheral cortex, surrounding and causing atrophy of cortical cells. The medulla is seldom affected.

Adrenal amyloid is always bilateral, except in very early stages.

It should be noted that the development of the perimedullary zone is subject to a sex difference because it is intimately related to the development and subsequent involution of the transitory or "X-zone" of the adrenal cortex. As described by Howard-Miller (1928) and by Deanesly (1928), this zone develops in early postnatal life in both sexes and in males regresses at around 4 weeks while in females it continues to grow. In mated females this zone degenerates during the first pregnancy while in virgins its regression starts at around 11 weeks. In some inbred strains this date may even be further postponed as reported by Daughaday (1941). The degenerating X-zone is replaced by loose reticular tissue with a rich capillary network, which separates the medulla from the final adult cortex. Because of the sex difference mentioned above, this perimedullary zone in adult mice is more marked in females than in males. As adrenal amyloid preferably infiltrates in this perimedullary zone, such infiltration is usually more extensive in female than in male mice of the same age and genetic constitution. Yet the total incidence, as distinct from the degree of amyloidosis, is similar in males and females, especially in the highest age groups. The condition is illustrated in figs. 5-10

III. Ovaries. Hyalinisation of the corpora lutea in ageing mice has been described by Fekete (1946) and by Loeb (1948). From their descriptions and from the illustrations of Fekete (1946) it is clear that these lesions are identical to those observed by ourselves (Thung e.a. 1956) but which we classify as amyloid for the reasons given in § 3. In fact, the corpora lutea are the main site of ovarian amyloid in mice. It starts in multiple patches which even in early stages enchroach upon the cytoplasm

of the corpus luteum cells, which is gradually replaced by amyloid material, nuclear remnants often being the last trace of the original structures. This probably led Fekete (1946) to assume a cellular process. Loeb (1948), however, described a vascular and perivascular origin, and in our own experience too early amyloid patches may be found surrounding capillaries within the corpus luteum. As this relation is not every-where demonstrable, further spread along intercellular spaces may be assumed (fig#. 11..). In extreme cases the corpora lutea may be transformed into round amyloid masses. ~~in which calcification may subsequently occur.~~ Fekete (1946) related the tendency to develop this "hyalinisation" to the age of the corpora lutea, while Loeb (1948) demonstrated that the age of the animal itself was also involved. Strain differences, indicating genetical factors, were noted by both authors and confirmed by our own observations (Thung e.a. 1955).

Amyloid deposits may also be found in the ovarian stroma, surrounding small blood vessels. At such sites small arteries may show involvement of the muscle cells of the tunica media. The origin of these deposits is not always clear. Besides remnants of former corpora lutea, atretic follicles probably contribute as amyloid infiltration has been observed in the theca interna of these structures.

IV. Testes. More than any of the other organs studied, the testes often show heavy vascular involvement not only of capillaries and small venules but also of small and medium sized arteries. In these, amyloid infiltrates in the tunica media, causing atrophy of the muscle cells. Further spread of the material through the interstitial tissue leads to atrophy an gradual disappearance of Leydig cells (fig#. 13...). Amyloid may also be found infiltrating between the collagenous fibres of the tunica albuginea,

especially where the latter is permeated by small blood vessels. Inside the basement membrane of the seminiferous tubules amyloid was never observed, which is consistent with the supposed avascular nature of these tubules.

V. Spleen. As amyloid infiltration in the spleen of senile mice is largely identical to that described in experimental amyloidosis, which is well documented (e.g. Kuczynski 1922, Letterer 1926, Jaffé 1926, Grayzel e.a. 1934), a short description may here suffice. The site of preference are the perifollicular venous sinuses. From patches in these parts, on the boundary of red and white pulp, the infiltrations develop into rings surrounding the follicles, while in extreme cases the red pulp is entirely replaced by amyloid. In such cases the lymphatic tissue is compressed into narrow strands surrounding the arteries. The arteries themselves are surprisingly little affected.

VI. Liver. For descriptions on amyloid in the liver we also refer to Letterer (1926) and other authors on experimental amyloidosis. The infiltrations start subendothelially in the portal venules and from here spread along the hepatic sinusoids, causing atrophy of liver cells. Usually, however, in senile mice there is little spread beyond directly periportal areas. Some authors report the central veins to be the site of preference in experimental amyloidosis (Grayzel e.a. 1934, Latvalahti 1953). It is possible, however, that differences in nomenclature are involved.

VII. Intestinal wall. Heavy deposits may occur in the stromal tissues of the intestinal wall. Dunn (1944) described infiltrations in the stomach, duodenum and colon. Varying in intensity, the condition probably extends throughout the entire length of the

intestinal canal. In our experience distal parts of the small intestine are more heavily affected than the duodenum . In the ileocoeal region the villous stroma often showed extreme amyloidosis (fig#..22..). The infiltration starts around capillaries and small venules, arterial vessels often remainig intact.

VIII. Heart. Amyloid infiltration observed in the heart of senile mice spreads along capillary and lymphatic spaces, surrounds and causes atrophy of muscle fibres, and leads to similar pictures as in human cardiac amyloidosis (Josselson e.a. 1952, Hüsselmann 1955). Contrary to the human condition, however, small and medium sized arteries sometimes were little affected, even in cases with marked pericapillary infiltration.

IX. Other sites. Amyloid was observed in many other organs of senile mice, on some of which, however, more observations are required. Thus in the pancreas widespread interstitial infiltration may occur, but involvement of the Langerhans islets has not yet been seen. The thyroid often shows heavy infiltration, leading to atrophy and necrosis of the follicular epithelium. The interstitium of the salivary glands may also be affected. In the central nervous system amyloid has as yet not been observed. It is often found between the layers of the myometrium, while in the vaginal wall small bloodvessels may be affected. In many of these organs the preference for capillary and venous vessels again was noted. Small and medium sized arteries may also be involved, but larger arteries and especially the aorta were always intact. Mention should be made of the frequent and heavy involvement of fat tissues. Here we find typical rimlike deposits covering the fat cells which are also known in human pathology, where they are sometimes held to be typical for primary amyloidosis (Dahlin 1949).

These deposits have been cited to corroborate a theory on epicellular genesis of amyloid (Peters 1921). In our opinion, however, they start around capillary vessels, their epicellular localisation being due to secondary spreading along lymphatic spaces ~~(figs. ...)~~.

One condition should be set apart as its inclusion among amyloid changes is dubious. In the skin, apart from involvement of subcutaneous fat and small blood vessels, heavy accretions may be found against the basement membrane of the cutis and the hair follicles, which are highly similar to the amyloid material. However, in old mice unaffected by amyloidosis this basement membrane is also often considerably swollen. In these latter cases this may be accounted for by the apposition of collagenous material, recognisable by heavy collagen staining reactions and marked birefringence. The relations between this latter process, and the condition in amyloidotic mice, in which the material stains lightly with collagen stains, are yet unknown.

5. Strain differences in the incidence and distribution of senile amyloidosis in mice.

The importance of genetical factors in the occurrence of amyloid in ageing mice was demonstrated by Dunn (1944) who reported striking differences in the incidence in 2 inbred strains and one group of hybrids. Heston and Deringer (1948) compared strains with high and with low incidence and their mutal f_1 and F_2 hybrids and backcrosses from the former to their parent strains. They found a recessive Mendelian inheritance of the tendency to develop amyloid in the duodenum, the spleen and the kidneys. Moreover, these lesions appeared to depend upon one common gene.

The following ~~tables~~ data may illustrate our own findings regarding the incidence and distribution of senile amyloidosis in some inbred strains and hybridisations. The data concern mixed groups of male and female mice, as sex differences were not found except of course in sex dependent organs as discussed above.

Table 1 demonstrates the age factor in the incidence of renal papillary amyloid in $(O_{20} \times DBA_f)F_1$ hybrids. All degrees of grossly recognisable papillonephritis were scored as positive, ranging from just an opaque papillary tip in either one or both kidneys, up to complete necrosis of the papilla with severe parenchymal atrophy. Cystic changes are uncommon in these hybrids. As the death rate below 20 months is negligible, the data up to the age of 18 months are on animals which were expressly killed for the purpose. Up to about 18 months the papillary changes were never severe, and destruction of cortical tissue was exceptional. Above 24 months, however, heavy papillonephritic lesions were frequent. In fact these kidney lesions are among the most frequent cases of death in these hybrids if allowed their

natural life span. The animals thus affected show marked signs of renal failure as polyuria, polydipsia, edema an ascites. On changes in renal function in papillonephritic mice we have reported elsewhere (Thung 1956).

Similar data on the adrenals are given in table 2, indicating the increasing frequency of microscopically detectable amyloid in these organs as the animals age.

The genetical factors involved are illustrated in table 3. These data concern mice of various strains and 2 groups of F_1 hybrids, all over 18 months old. It should be noted that here, as in the former tables, there was a parallel between the frequency and the severity of the lesions in various groups. In O_{20} and CBA mice heavy degrees of parenchyme destruction were never seen, while old ($C_{57}BL$ x DBA)F_1 hybrids usually are heavily affected. In this latter group cystic dilatation of tubules and glomerular capsules is frequent. $C_{57}BL$ and A mice hold midpositions between the hybrids and the other inbred strains. It is remarkable that the incidence of papillonephritis in strain A is much lower than reported by Dunn (1944) or by Gorer (1940). Such differences are probably in part ascribable to environmental differences. Heston e.a.(1945) found that changes in the composition of the diet influences the frequency of amyloid lesions in strain A.

Our findings on the incidence in F_1 hybrids and their parent strains seem to disagree with the conclusions of Heston and Deringer (1948) regarding a recessive inheritance. Yet we think that the findings of these authors and of ourselves are not necessarily contradictory, for while renal amyloid is seldom found in old DBA_f mice, these animals have a high incidence of amyloid infiltration in other organs. Apparently not only the incidence of amyloidosis shows strain specificity, but also the distribution

of the amyloid material in the body is genetically conditioned. This finding, which of course complicates genetical deductions is illustrated in ~~table 4~~ figure 23. This ~~table~~ figure gives data on some organs in which amyloid was diagnosed microscopically. ~~The percentages of amyloid infiltration are minimum values, as serial sections were not made. In most organs this value will approximate the true value because amyloid is distributed rather evenly as described in § 4. In the ovaries, however, a much higher true incidence is to be expected and was in fact found in groups of serially sectioned ovaries, because amyloid is often confined to the corpora lutea.~~ In this material too, the frequency ran parallel to the severity of the lesions. It appears that DBA_f mice, in which no grossly recognisable renal amyloid was found (table 2), showed marked amyloid infiltration of the adrenals and the ovaries.

Comparable data from other authors are the reports by Gorer (1940), Dunn (1944), and Heston and Deringer (1948) on the frequency of renal lesions in ageing mice of various strains. Gorer (1940) described papillary necrosis in strain A and strain $C_{57}BL$, and glomerular "hyalinization" in the latter strain only. Both lesions are histologically identical to what is here described as amyloid. On the ovaries there are reports by Fekete (1946) and by Loeb (1948). Fekete (1946) observed "hyalinization" of the corpora lutea in DBA mice and ascribed this condition to a delayed involution of the corpora lutea in this strain. She did not find it in $C_{57}BL$ ovaries. Loeb (1948) found "hyalinization and gelatinization" in the corpora lutea as well in interstitial tissues of the ovary in 8 inbred strains including $C_{57}BL$. Data on strain differences in the frequency of adrenal amyloid are given by Blaisdell e.a. (1941).

From the observations reported here we may conclude that in senile mice a diversity of genetically conditioned patterns of amyloid distribution exists. Of course many more data would be required to map out these various patterns in different inbred strains. Yet it is clear that, while all strains observed thus far are potentially liable to develop amyloid infiltration in old age, in some the distribution and severity of these lesions are such as to constitute a major cause of death, while in others amyloid deposits are incidental and relatively insignificant.

6. Relation to experimental amyloidosis in mice.

Just as of amyloid formation in general, the pathogenesis of the amyloidosis described here in senile mice is as yet unknown. However, its position as a pathological concept may be clarified by a discussion of its relation to experimentally induced amyloidosis in mice. The similarity of both conditions is apparent from the histology of the organs involved. As early authors like Davidsohn (1897) were interested only in whether amyloid induction was successful or not, Letterer (1926) is among the first to give adequate histological details on experimental amyloidosis in mice. His observations on the staining reactions and on the histology of amyloid in the spleen, the liver and the kidneys are in complete agreement with our findings as reported above, and so are those of Smetana (1925), Jaffé (1926) and others. Dunn (1944) noted that these authors in early stages report crystalline deposits which she did not observe in untreated mice. In our experience the vague brush- and needle-like outlines described by these former authors may scmetimes be found in senile amyloidosis too (fig. 12). It has been claimed for human amyloid that the impression of crystalline structure is occasioned by reticular fibres embedded in the amyloid rather than by this substance itself (Missmahl & Hartwich 1954). In the case of early experimental deposits the suggestion of Baily (1916) that processes of fixation etc. are responsible for these structures, seems more probable. In our opinion this may be true also for similar structures observed in senile amyloidosis.

Another apparent difference is the fact that experimental amyloidosis is often localized in the spleen only. The liver and the kidneys may also become involved, but further spread is seldom reported. This has led to the suggestion by Dunn (1944, 1948) that the relation between experimental and spontaneous

amyloid in mice might be similar to that between secondary and primary amyloidosis in man. Heston and Deringer (1948), working on the genetics of spontaneous amyloidosis in ageing mice, carefully excluded from their material all animals with amyloidosis ascribable to cutaneous abscesses, dermatitis etc. In such animals the spleen was found to be more often affected than in mice with the so-called primary type of amyloidosis.

Now in human pathology it is recognized that the distinction between primary and secondary amyloidosis is somewhat artificial as there is much overlapping of these conditions and many cases are not strictly classifiable under either heading. Secondary amyloidosis is often found to be "primary" in its distribution or staining reactions, while primary amyloidosis may often show localisations of the "secondary" type, as is apparent from the review of Mathews (1954). King (1948) has aptly argued that the immediate cause of the amyloid infiltration is just as unknown in secondary as in primary amyloidosis. This classification merely marks our acuteness in discerning conditions which play some part in the probably complicated causal chain leading to amyloid deposition. With progress in research, such classification therefore will have to be altered. Such progress is illustrated by the recognition of chronic rheumatoid arthritis as a condition which prediposes to amyloidosis. While quite recently this relation was only hesitantly suggested (Trasoff e.a. 1944), it is now increasingly recognised (Gedda 1955) and even plays an important role in modern theories on the mechanism of amyloid formation (Teilum 1952). Thus the range of conditions which are known as possible causes of "secondary" amyloidosis is gradually widening. Another illustration of the arbitrary nature of such classification are the amyloid deposits in cases of multiple myelomata. Here the distribution and staining properties usually are of a "primary"

type, and the "secondary" nature of the condition was not recognised up to 1931 (Magnus-Levy). At present, several varieties of "primary" amyloidosis gradually emerge, such as cardiac amyloid related to old age (Hüsselmann 1955), amyloid with nervous system involvement related to genetical constitution and dietary factors (da Silva Horta 1955), and familial amyloidosis with renal, splenic, hepatic and adrenal involvement (Ostertag 1950). In short, former subdivisions become increasingly untenable and the term amyloidosis now comprises a broad spectrum of conditions which are highly variable in many respects, but between which gradual overlapping and many transitional forms are found.

Similarly in mice, we think a strict division between secondary and primary amyloidosis, as advocated by Heston and Deringer (1948), is impracticable for the following reasons. Experimental amyloid induction in mice is a very unspecific process. It has been realised since the work of Davidsohn (1897) that mice are exceptionnally susceptible to amyloidosis, which is the very reason why they are the experimental animals of choice in amyloid research. Other animals used for this research include rabbits and chickens. In some species, however, amyloid formation is very difficult to induce. Loeschke (1927) and Graygell (1934) using casein injections, got no amyloidosis in rats. Early experimentors found that caviae were refractory to amyloid induction (cf. the review by Lubarsch, 1897), and only quite recently has the first successful attempt on this species been reported (Pirani e.a. 1949). Mice, however, show a very ready tendency to amyloid development which is apparent also from the wide range of procedures by which amyloidosis may be induced in these animals. To cite a few: after the injections of bacterial cultures, dead or alive, of early authors (Davidsohn 1897, Frank 1920, Domagk 1924) came the administration of casein

(Kuczynski 1922) which is now universally practised. Other protein products, however, like gelatin and egg protein (Letterer 1926) did the trick as well and so did inorganic substances like colloidal sulfur (Letterer 1926), phenolic and saline solutions, and even destillated water (Letterer 1934). Meanwhile it was found that amyloidosis in mice may also be caused by tar painting (Döderlein, 1926. Also Twort and Twort, 1932, who use the term hyaline disease), or by grafted tumours (Lubarsch 1910). Amyloidosis in cysticercus-affected mice (Benassi and Baggi 1952) also belongs in this class of incidental findings. In experimental amyloid research the usual procedure at present is to give a standard series of casein injections as basic treatment. Additional treatment is then superimposed and the depressing or enhancing effect upon amyloid induction is observed. In this way an amyloidgenic effect was found to be exercised by increased room temperature (Perasalo 1949), cortisone or ACTH (Teilum 1952), nitrogen mustard (Teilum 1954), vitamin C deficiency and TSH (Uotila e.a. 1955).

The common factor discerned in all these procedures varies with the advocated theory on amyloid formation. It has been concluded that any agent disturbing the collodial stability of serum proteins may occasion processes of protein breakdown leading to disturbed antigen-antibody relations and amyloid formation (Letterer 1934). Recently Letterers theory on antigen-antibody relations in connection with amyloid is being reformulated in terms of exhaustion of antibody-forming potencies of mesenchymal (reticulo-endothelial) cells (Gössner e.a. 1951, Latvalahti 1959) or of a perversion or suppression of the normal metabolism of plasma cells (Teilum 1952, 1954). However this may be, for the present purpose it suffices to note that in mice such a diversity of procedures leads to identical or closely related amyloidotic conditions. Apparently the treshold to amyloid formation,

whatever its mechanism, in mice is so low as to make amyloidosis a quite unspecific reaction. Of this readily elicited reaction, the condition in untreated ageing mice in our opinion is just another manifestation.

If this is true, no strict separation should be found between experimental and senile amyloidosis in mice. Yet experimentally induced amyloid shows a more restricted range of distribution and is usually concentrated in the spleen and the liver. Senile amyloidosis on the other hand affects practically all organs, and the involvement of spleen and liver may be relatively slight. However, the different distribution in experimental amyloidosis may reflect the technique of the experiment rather than the nature of the degenerative process. When amyloidogenic agents are given orally, the lesions may be confined to the liver , as found by Murata and Yoshikawa (1927) feeding mice with various forms of silicate. Using subcutaneous injections, short term experiments disclose involvement of the spleen only. Later the liver and the kidneys may be affected too(Letterer 1926). In protracted experiments, however, other organs become involved and the distribution becomes increasingly similar to that of amyloid in untreated old mice. Thus Jaffé (1926), administering daily casein injections for up to 100 days, found involvement of intestine, heart and ovaries in his oldest groups. It appears that experimental amyloidosis, which initially may reflect the method of its induction, in chronic forms highly resembles the senile amyloidosis of untreated mice. Another indication of the basic similarity of these two conditions is the observation by Smetana (1925) that in old mice amyloid induction succeeds more rapidly than in young animals. Moreover, just as in senile amyloidosis (Heston e.a. 1945, Heston and Deringer 1948), experimental amyloidosis also depends upon genetical and dietary conditions. Letterer (1934)

stressed that different breeds of mice may react variably to the same treatment, and several authors have demonstrated the influence of dietary factors upon the incidence of casein induced amyloidosis (Letterer 1934, Grayzell e.a. 1934, Ku and Simon 1934).

Summarising we may conclude that, just as in man, we find in mice a wide range of conditions between which gradual transitions exist and which therefore, and because of their common histological characteristics, are ranged under the term amyloidosis. Such amyloidosis may be elicted in peracute forms as in the experiments of Domagk (1924) and Letterer (1926) who claimed to have seen amyloid within some minutes after intravenous injection of bacterial suspensions and protein solutions respectively. It may also develop more chronically as in the experiments with repeated subcutaneous casein injections, or in animals weakened by tar painting, grafted tumours etc. as cited above. Finally it may occur also, in forms undistinguishable from these latter conditions, in untreated ageing mice. Here we illustratively use the terms "senile" and "spontaneous" amyloidosis, well realising that of course various factors will be found to contribute to the causation of this condition. Of these, some are known already: genetical make up and dietary habits of the animals. Other factors, like environmental temperature and humidity, enteric and cutaneous parasites etc. remain to be analysed. Yet the fact that these amyloid infiltrations accumulate with such regularity in untreated old mice, increasing in both frequency and severity as the animals age, entitles us to call it a form of senile degeneration. For what else is senescence, biologically speaking, but the gradually impairing efficiency of the organism in the interplay of genetical and environmental processes.

7. Senile amyloidosis in man.

As by senile amyloidosis we understand the occurrence of amyloid deposits in aged subjects without one of the well known causative diseases, in the discussion of this subject mention should be made of the concept of primary amyloidosis in general. The idea (not the name) of senile amyloidosis stems from Soyka (1876) who mentioned the unexplained occurrence of widespread, though often slight amyloid deposits in aged subjects, often with special preference for the heart. He specified the cases of an 80 years old female and an 81 years old male subject. In those days, however, the discussion around amyloid was entirely dominated by its causal relation to tuberculosis and other bacterial processes, and even in 1904 Schmidt waved aside the notion of senile or other "spontaneous" forms of amyloidosis. In his opinion such cases should be ascribed to chronic enteric infections or other bacterial diseases.

The concept of amyloid deposits without known cause became firmly established since the paper of Lubarsch (1929) who cited 11 cases up to then reported and described 3 of his own. These cases were distinghuished from classical amyloidosis by

1. Atypical localisations, the spleen and liver being unaffected while for instance the lungs, muscles, intestinal wall and the heart often were involved.
2. Atypical staining reactions, especially with the iodine methods.
3. Absence of any of the known causative diseases.

Lubarsch regarded these cases as intermediary forms between classical generalised amyloidosis and local tumourous amyloid deposits. It is noteworthy that he already desisted from detailed classification because of the fundamental ignorance on the nature of amyloid infiltration. Since then a large number of cases complying to the characteristics mentioned by Lubarsch (1929) has been reported.

In German literature the name paramyloidosis, stressing the atypical localisation and staining reactions is most frequently used. Anglo-Saxon authors usually speak of primary amyloidosis which emphasises the unknown causal relations. This difference, and the fact that,moreover, the application of both terms varies with the personal view of the authors on the classification of amyloidosis, account for some variability in the material covered by various reviews given through the years. However, to mention a few: while Lubarsch (1929) mentioned 14 cases, Koletsky and Stecher in 1939 found 24 cases, while Dahlin in 1949 found 57 reported cases of primary systemic amyloidosis. Five years later this number had increased to 98 (Mathews 1954), and the latter author therefore observes that primary systemic amyloidosis is being identified with increasing frequency. This increase will be even more marked if recently described varieties are taken into account, such as the familial paramyloidosis with periferal nervous involvement of which over 100 cases are known in Portugal (da Silva Horta 1955) or the familial primary systemic amyloidosis on which clinical observations are at present being made in the United States (Block e.a. 1955, Jackson e.a. 1955).

For the present purpose two observations are relevant, the first of which is the high incidence of cardiac amyloid in primary systemic amyloidosis. Mathews (1954) reported myocardial localisations in 72 of 98 cases, the next organ in frequency of involvement being the spleen with 40 cases. Clinically too, cardiac symptoms are often dominant (Lindsay 1946, Mathews 1954).Secondly, it has been found that in other cases, which are often not included in the reviews on systemic primary amyloidosis, the heart is the dominant or even the only site of atypical amyloid infiltration, and that this usually concerns old patients. Thus King (1948) reported 5 patients with atypical amyloidosis confined to the heart

and the lungs, which were all over 83 years old, the average age being 87,8 years. For comparison we may mention that in Mathews' review (1954) the average age of all cases of primary systemic amyloidosis was 55,4 years. More data on the occurrence of primary amyloidosis in the heart in aged persons were given by Josselson e.a. (1952), while recently this subject was reviewed by Hüsselmann (1955) who himself found a 15 % and 10 % incidence respectively in males and females over 70 years old. Though the heart in these cases is the main site of amyloid infiltration, small bloodvessels in other organs often are also affected and Hüsselmann (1955) points out that there is no sharp distinction between this senile cardiovascular amyloidosis and the cases of generalised atypical amyloidosis with heavy carddovascular involvement. Incidentally, this conclusion is a substantiation of Soyka's (1876) tentative remarks! In short we may conclude that the heart is more than other organs disposed to atypical amyloid infiltration, as is apparent from its frequent participation in cases of generalised primary amyloidosis and also from its solitary involvement in cases where other organs are little affected. In these latter cases old age emerges as a factor in the as yet largely unknown pathogenesis of the condition.

In this context some other reports relating amyloid to ageing should be mentioned. In 1927 Divry asserted that the substance occurring in the brain in senile plaques and in the lesions of Alzheimers disease are identical or similar to amyloid. This observation has been supported or opposed by several authors, as cited by Missmahl e.a. (1954). Recently Divry (1956) has summarized his findings regarding cerebral amyloid in aged subjects. Besides senile plaques and Alzheimer lesions, this includes changes in meningeal vessels and pericellular deposits in the choroid plexus.

In the skin amyloid deposits in relation with old age have been observed by Freudenthal (1930) and by Love (1952). In these cases, however, other pathological processes were also involved. Love (1952) regarded the amyloid changes as an expression of metabolic disturbances in the ground substance caused by cutaneous arteriosclerosis.

Gellerstedt (1938) claimed that the so-called hyaline degeneration of Langerhans' islets in the pancreas constitutes a local amyloidosis. This lesion usually occurs above the age of 50 years, and is not necessarily accompanied by diabetes. Gellerstedt (1938), whose observations were confirmed a.o. by van Beek (1939) and Arey (1943), described pericapillary deposits in the islets which gave positive amyloid staining reactions and which caused atrophy of the islet-cells. Gellerstedt (1938) found a 46,3 % incidence in a series of 110 autopsies on persons between 50 and 90 years including only 3 diabetics. Van Beek (1939) found 12,5 % insular amyloid in 56 non-diabetics over 30 years, Arey (1943) 16,6 % in 114 non-diabetics over 50 years old. The latter authors suggest a relation with sclerosis of small pancreatic vessels, such as is also described by authors which do not recognize the amyloid nature of these lesions (Moschcowitz 1956). Gellerstedt (1938) found no relation between insular amyloid and vascular sclerosis and describes the condition as an instance of senile amyloidosis.

Another instance is the condition described in the seminal vesicles by Bursell (1941). In generalised amyloidosis the seminal vesicles may show vascular and perivascular infiltration. In cases of isolated amyloidosis of the seminal vesicles, however, the material occurs in strands and patches directly beneath the epithelium, as has also been described by Lubarsch (1930). Bursell (1941) found that this latter condition is

fairly frequent in older subjects, a 16,9 % incidence being found above the age of 50 years. The frequency between 76 and 90 years (34,2 %) was significantly higher than between 46 and 60 years (7,7 %). The author discussed and excluded possible causes like inflammation and plasma-cell proliferation, and suggests some unknown age-dependent change in local protein metabolism.

In passing mention may be made of 2 cases of isolated amyloidosis and necrosis of the renal papilla in aged patients with pyelonephritis (Mellgren and Redell, 1940). The authors discuss the possible relation with senile amyloidosis, but definite conclusions could, of course, not be drawn from these rare cases.

Summarising we may conclude that in human pathology there are several indications of amyloidogenic tendencies in old age. Best documented is the case of cardiac amyloidosis (Hüsselmann 1955). This condition merges into the well established pathological entity of primary systemic amyloidosis with heavy cardiac involvement. The latter disease may be regarded as a more extensive and often presenile expression of the senile condition analysed by Hüsselmann (1955). Whether senile cardiac amyloidosis and the other lesions mentioned above occur combined or independently is unknown, because systematis combined investigations on all organs concerned have not yet been done. It is possible that various patterns of senile amyloidosis exists, comparable to the strain differences found in senile mice (§ 5). Mention should be made of the form of generalised amyloidosis frequent in Portugal which in some respects shows precocious and extreme manifestations of lesions which in slighter forms may occur as a senile condition. In this disease, which probably is genetically conditioned, widespread amyloidosis is found at young ages, with involvement of the peripheral nervous system. The heart is also heavily infiltrated, and it is of interest that in the central nervous system lesions similar to

senile plaques were observed. Infiltration of pancreatic islets, however, was as yet not observed, and in the seminal vesicles the amyloid deposits were of the perivascular type (da Silva Horta 1955).

Finally it should be stressed that the use of the term senile amyloidosis does not imply any causal explanation. It only indicates that in these conditions the as yet unknown aetiological factors become operative or manifest in old age. That these factors themselves are not strictly age-dependent is demonstrated by the forms of early "primary" amyloidosis cited above. In this respect too, the situation is similar to what is found in mice, for in this species the experimentally induced amyloidosis may be regarded as a precocious variety of the senile condition.

8. Comparable conditions in other animals.

On senile amyloidosis in other animals than in mice little is known. This is not because no amyloid occurs but rather because the age of the animals concerned often is unknown. Moreover, reports on old animals generally are scarce, while finally in many species it is uncertain at what age the term senile ~~amyloid~~osis is applicable, as data on longevity are lacking. The follow~~ing~~ data may, however, be relevant to the present theme.

In a review on amyloidosis in animals, Hjärre (1933) mentions 11 cases of amyloidosis in dogs, in 6 of which no causative disease was found. Most of these animals were of advanced age, and the kidneys were site of preference, though the liver, the spleen, and in a few cases the adrenals were also involved.

The same author (Hjärre,1933) describes some cases of primary amyloidosis in horses, with marked cutaneous deposits. Apparently spontaneous amyloidotic lesions similar to the well known condition in horses used for the production of antisera, were formerly known to occur in certain regions of Russia (Arndt 1928).

In cattle, amyloidosis, mainly of the kidneys, is quite frequent. The animals are usually of advanced age, and while the condition often is ascribable to mastitis or other diseases, in some cases no causative disease is found (Primgaard 1930, Hjärre 1933).

Other data from Hjärre's (1933) paper include an old otter with generalised amyloidosis, no cause being stated, and a 14 years old tomcat in which enteritis and scabies were held to have caused amyloidosis. In this connection the case of Rubarth (1935) may be mentioned who found amyloid in the islets of Langerhans and in small pancreatic arteries in a 10 years old tomcat, no other organs being affected.

Primary amyloidosis has been reported as a cause of death in tenrecs (Osman Hill 1955). The liver showed heavy deposits and the kidneys were also involved.

Gillman and Gilbert (1955) give extensive descriptions of primary systemic amyloidosis in 4 female baboons. The age of these animals varied between around 6 and 13 years. It is of interest that in these cases there was marked cardiovascular involvement. The authors discuss the etiological possibilities and suggest that in this species cyclic transport and redistribution of interstitial proteins, associated with the cyclic swelling and subsequent involution of the sex skin of the female animals, may involve a disposition to disturbances in protein metabolism of which amyloid deposits may be the outcome.

From these random data it appears that in various animals amyloidosis may be found without known causative disease, and that this condition is often not sharply demarcated from secondary amyloidosis, cf. the cases of dogs, horses and cattle as cited above. Here too, the term primary amyloidosis just indicates our ignorance of the causal relations in such cases .Finally here too indications are found, though slighter than in human and mouse pathology for the reasons stated above, that old age may in some of these cases be involved in the as yet unknown etiology.

9. Conclusions.

We have described and cited instances where in ageing organisms unexplained derangements of protein metabolism led to infiltrations in various tissues for which amyloidosis is the only fitting diagnosis.

It has been pointed out that the term amyloid, though often instinctively associated with the disease secondary to chronic tuberculosis etc., in fact covers a wide spectrum of conditions of varied and partly unknown etiology. Though the anatomical distribution and the chemical characteristics of the deposits in these conditions are highly variable, the common histological characteristics and the existence of many transitional forms between the different types of amyloid disease, to some degree justify the use of a common denominator. This is necessitated, moreover, by the present lack of a more discriminating nomenclature, due in part to the limitations of our routine optical and tinctorial methods.

Of the various types of amyloidosis some are distinguished by the combination of unknown etiology and a high age of the subjects concerned. That this combination is far from rare, is well established in man and in mice, while in other animals its occurrence is probable too. To these cases, in which as yet unknown causal factors are somehow linked to processes of ageing, the term senile amyloidosis is applicable. In its development, as in any biological or pathological process, genetical factors of course play a part. Genetical differences, after all, cause mice to develop such conditions after 2 and men after 80 years. Genetical factors too are involved in the anatomical distribution of the substance in different strains of mice or different human inviduals. Genetical factors, finally, cause mice to be so much more liable than other laboratory mammals or man to develop amyloidosis, whether

after experimental treatment or "spontaneously" in old age.

In breeding in mice has provided us with some strains in which the tendency to develop senile amyloidosis is genetically so concentrated as to constitute a major cause of death, while in other strains such tendencies are practically eliminated. It is to be expected that comparative studies on such strains will help to analyse the etiology of senile amyloidosis and the various forms it may assume. This will be quite consistent with the leading role which mice have had in amyloid research since 1897.

10. Summary.

An analysis of the general histological and tinctorial qualities of widespread protein deposits of unknown causation in old mouse tissues, resulted in the diagnoses amyloid infiltration (§ 3). The histological characteristics of this infiltration are described for various organs (§ 4), and data are given on the frequency of the lesions in some inbred strains of mice (§ 5). It appears that in some strains amyloidosis is a major cause of death in old age, while in others the findings are slight and incidental only. The relations between this senile amyloidosis and experimentally induced amyloidosis in young mice are discussed (§ 6), and it is concluded that no strict distinction between both conditions exists and that the latter may to some degree be regarded as a precocious form of the former.

Similar relations may be found ~~to exist~~ in other species: various forms of senile amyloidosis in man are discussed, and it is pointed out that some of these are closely related to certain forms of primary systemic amyloidosis at younger ages (§ 7). The relations between primary and secondary amyloidosis are also discussed and it is concluded that the term amyloidosis covers a wide range of different conditions which are linked, however, by various transitional forms. In this range senile amyloidosis has its place as a variety in which as yet unknown causal factors are linked to processes of ageing. Such senile amyloidosis occurs in man, in mice, and probably in other animals too (§ 8), but in mice it is more frequent and more amenable to research than in other mammals (§ 9).

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Table 1.

Incidence of grossly demonstrable renal amyloidosis in (O_{20} x DBA_f)F_1 hybrids at various age levels.

Age:	Number of mice observed:	Percentage showing gross papillonephritis or papillary amyloid infiltration in one or both kidneys:	
0- 6 m.	25	0 %	
6-12 m.	20	20 %	Killed experimentally.
12-18 m.	10	20 %	
18-22 m.	6	50 %	
22-24 m.	21	86 %	
24-26 m.	52	87 %	Died or killed
26-28 m.	28	96 %	in extremis.
28-30 m.	14	100 %	

Table 2.

Incidence of amyloid in the adrenals of (O_{20} x DBA_f)F_1 hybrids at various age levels.

Age:	Number of mice observed:	Percentage with adrenal amyloidosis:
0- 6 m.	7	0 %
6-12 m.	10	0 %
12-18 m.	13	31 %
18-22 m.	-	-
22-24 m.	13	54 %
24-26 m.	38	76 %
26-28 m.	46	87 %
28-30 m.	25	96 %

Table 3.

Incidence of grossly demonstrable renal amyloidosis in inbred mice over 18 m. old.

Strain or hybridization:	Number of mice observed:	Percentage showing gross papillonephritis or papillary amyloid infiltration in one or both kidneys:
O_{20}	65	17 %
$(O_{20} \times DBA_f)F_1$	121	88 %
DBA_f	25	0 %
$(C_{57}BL \times DBA_f)F_1$	25	8½ %
$C_{57}BL$	108	56 %
CBA	58	5 %
A	44	48 %

Legends to figures.

Fig. 1 Normal renal papilla in an adult (9 m.) mouse.
(Strain O_{20}, haematoxylin azophloxin, 200x)

Fig. 2 Amyloidotic renal papilla in a senile (25 m.) mouse.
(Hybrid $F_1(O_{20}xDBA_f)$, Mallory's connective tissue stain, 150x)

Fig. 3 Mouse kidneys cut longitudinally and stained in dilute Lugol's solution.
(Hybrids $F_1(O_{20}xDBA_f)$, 2,5x)
1 : Young (5 m.) mouse with normal renal papilla.
2-4: Old mice (20-26 m.) with papillary amyloidosis and increasing degrees of "papillonephritis".
2 : No gross cortical lesions.
3 : Cortical atrophy and small glomerular capsule-cysts
4 : Severe cortical atrophy.

Fig. 4 "Papillonephritis" in a 24 m. old mouse. Amyloidotic papilla and wedge-like cortical atrophy with some glomerular capsule-cysts.
(Hybrid $F_1(O_{20}xDBA_f)$, Mallory's connective tissue stain, 11x)

Fig. 5 Amyloid infiltration in the adrenal of a 25 m. old male mouse.
(Strain $C_{57}BL$, haematoxylin azophloxin, 150x)

Fig. 6 Corticomedullary junction in normal adrenal of an adult (10 m.) male mouse. Medullary side marked M.
(Hybrid $F_1(C_{57}BLxDBA_f)$, haematoxylin azophloxin, 600x)

Fig. 7 Corticomedullary junction with incipient amyloid infiltration in a senile (26 m.) male mouse.

Compression and atrophy of cortical cells.
(Hybrid $F_1(O_{20}xDBA_f)$, haematoxylin azophloxin, 600x)

Fig. 8 Amyloid infiltration in the adrenal of a senile (28 m.) female mouse.
(Hybrid $F_1(O_{20}xDBA_f)$, haematoxylin Congo red, 150x)

Fig. 9 Corticomedullary junction with perimedullary reticular zône without amyloid in a 20 m. old female mouse.
(Strain $C_{57}BL$, haematoxylin azophloxin, 600x)

Fig. 10 Perimedullary reticular zône with incipient amyloid infiltration in a 25 m. old female mouse.
Pericapillary amyloid leaves cortical cells intact.
(Hybrid $F_1(O_{20}xDBA_f)$, haematoxylin azophloxin, 600x)

Fig. 11 Amyloid infiltration in a corpus luteum of a 27 m. old mouse. Compression and atrophy of lutein cells.
(Hybrid $F_1(O_{20}xDBA_f)$, haematoxylin azophloxin, 320x)

Fig. 12 Crystalloid patch of amyloid in testicular interstitium of a 26 m. old mouse.
(Hybrid $F_1(O_{20}xDBA_f)$, haematoxylin azophloxin, 1500x)

Fig. 13 Amyloid infiltration of testicular interstitium in a 27 m. old mouse. Amyloid causes compression and atrophy of Leydig cells. Small arterioles are involved, while segment of seminiferous tubule is shown free of amyloid.
(Hybrid $F_1(O_{20}xDBA_f)$, Mallory's connective tissue stain, 600x)

Fig. 14 Incipient amyloidosis in the spleen of a 23 m. old mouse. The distribution is typically perifollicular, as contrasted to the usual pattern in human amyloidosis.
(Strain $C_{57}BL$, haematoxylin azophloxin, 150x)

Fig. 15 Segment of an amyloidotic follicle in the spleen in a human case of pulmonary tuberculosis with generalised secondary amyloidosis. Amyloid surrounds the follicular artery and infiltrates the lymph follicle, as contrasted to the follicular localisation in mice.
(By courtesy of Prof. Th. van Rijssel, Laboratory of Pathological Anatomy, State University, Leiden. Haematoxylin eosin, 150x)

Fig. 16 Remnant of a lymph follicle in heavily amyloidotic spleen of a 29 m. old mouse, showing intact follicular artery. (Hybrid $F_1(O_{20} \times DBA_f)$, combined elastin van Gieson stain, 600x)

Fig. 17 Amyloid infiltration in the liver of a 24 m. old mouse. Amyloid typically surrounds the portal venules, as contrasted to the pattern in primary amyloidosis in man. The hepatic arteries are not discernible at this magnification. (Hybrid $F_1(C_{57}BL \times DBA_f)$, haematoxylin azophloxin, 150x)

Fig. 18 Liver from a case of human generalised primary amyloidosis. The hepatic arterioles show amyloid infiltration while the portal venules remain free. (By courtesy of Prof. Th. van Rijssel,. Haematoxylin eosin, 150x)

Fig. 19 Detail of periportal area in the liver of a 24 m. old mouse, showing subendothelial amyloid in the portal venule, while the hepatic artery remains intact.
(Hybrid $F_1(O_{20} \times DBA_f)$, combined elastin Congo red stain, 600x)

Fig. 20 Normal duodenal villus of a 4 m. old mouse.
(Strain A_f, Mallory's connective tissue stain, 600x)

Fig. 21 Amyloidotic duodenal villus of a 29 m. old mouse. (Hybrid $F_1(O_{20} \times DBA_f)$, Mallory's connective tissue stain, _600x)

Fig. 22 Ileum of a 28 m. old mouse with heavy amyloidosis of the villous stroma. (Hybrid $F_1(O_{20} \times DBA_f)$, haematoxylin Congored, 150x)

Fig. 23 Strain differences in organ distribution of senile amyloidosis. Data from groups of mice over 18 m. old.