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On the Pathogenesis of Amyloidosis

By

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With 4 Figures in the Text

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Up to the present time there is no common concept as to the ways amyloid is formed and deposited in tissues. Its presence in the immediate vicinity of the capillary endothelium along reticular fibers serves as the basis for some workers considering the formation of amyloid to be consequent on the infiltration of plasma proteins and glycoproteins (DICK, LEITER 1937, LARSEN 1957, WAGNER 1955). A factor favoring this viewpoint is the hypoproteinemia seen in amyloidosis.

Because amyloidosis is associated with chronic infection accompanied by tissue decomposition and occurs after a long-term immunization of horses LOESCHKE (1927) then LETTERER (1934—1953), postulated that amyloid is a product resulting from an "antigen-antibody" reaction. Another factor in favor of this viewpoint is the increase in the globulin, observed in amyloidosis, particularly plasma gamma-globulins (KERNER, FRENKMAN 1935, EKLUD, REIMANN 1936). There are, however, indications that amyloid is not just a simple product of antigen and antibody precipitation in tissues. Thus, GILES and CALKINS (1958), who produced casein amyloidosis in rabbits, stressed the certain disparity between the titer of antibodies against the casein and the content of amyloid in organs. Taking this as a basis, it is the authors' opinion that in the course of the formation of amyloid, apparently non-precipitating antibodies or some other immunological factors are of definite importance. This point of view is also shared by a number of other research workers (BRAUNSTEIN, BUERGER 1959, CALKINS, COHEN 1958).

Some experimental data recently were obtained bear witness to the fact that amyloid formation is coupled with a deranged protein-synthetizing function of the reticulo-endothelial system (APITZ 1940, TEILUM 1954—1961). In this connection TEILUM (1961) distinguishes two phases in the development of amyloidosis: on the one hand, an active (preamyloid) phase characterized by proliferation of the reticulo-endothelium, by the appearance of pyroninophilic cells, and by an increased level of blood plasma γ-globulin and on the other hand, an amyloid phase proper, typified by suppression of cellular transformations in the reticulo-endothelial system, by a drop of the γ-globulin level, and by a rise of the α_2- and β-globulin fractions of the blood serum. The transition from the active phase to the amyloid phase is attended with a stimulation of immunological mechanisms.

Hence, not one of the now accepted points of view on the pathogenesis of amyloidosis has so far been definitely confirmed.

The present work is an attempt to form a concept of the genesis of amyloidosis in an experiment, taking as a basis the analysis of biochemical, pathomorphological and immunological results.

Materials and Procedure

A 10 per cent casein solution in saline was injected subcutaneously into 30 rabbits in doses of 5.0 ml three times a week (2.0 g of novocain and 300.000 units of penicillin were added to each 100 ml of the casein solution). A control group included 20 animals. The rabbits were fed on a standard diet with of water ad lib.

At regular intervals during the experimental period from 6 to 80 days biochemical changes were investigated in the blood and urine. Further data were obtained by pathomorphological studies, and by the complement fixation test involving organ and serum proteins.

Chemical Methods. The total serum protein was measured spectrophotometrically. To register biochemical changes in the blood paper electrophoresis was employed. This was done on the Leningrad paper of the "B" brand at room temperature, 400 V, amperage — 10 mA, in a veronal buffer at pH — 8.6 for 6 hours. Proteins were stained with an acid blue-black stain; glycoproteins by the Ceriotty method (1957). Electrophoregrams were evaluated on a self-recording microphotometer MF4. The protein level in the urine was determined according to STOLNIKOV.

Pathomorphological Methods. These investigations included the spleen, lymph nodes (mesenteric), kidneys, liver, adrenal glands, intestines, heart and lungs. The materials were fixated in formalin and acetone. Common methods of staining were employed (with hematoxylin-eosin, connective tissue — according to van Gieson and Mallory, elastic fibres — according to Weigert and argyrophil fibres — by Tibor-Papp), staining for amyloid (Congo red and methyl violet), histochemical methods (RNA — by Brachet, DNA — by Feulgen, PAS-reaction, toluidine blue, fibrin — by Weigert, hyalin and fibrinoid, according to Endesch, Sudan III and Goldman's method), along with histoenzymatic ones (ribonuclease, diastase, lidase, collagenase). In addition to this polarization microscopy was made use of (non-stained sections and those stained with Congo red).

Immunological Methods. The complement fixation test (CFT) was performed by the usual method according to Bordet and Gengou. Alkali-soluble protein fractions of the spleen, kidneys and liver were used as antigens, obtained by the methods of HASS and SCHULZ (1940), specially devised for the isolation of amyloid proteins from amyloid organs. The reaction was carried out with the blood serum involved from the animals whose organs were examined. The serum was inactivated in a water bath at 60° for 30 minutes and then made ready for dilution at 1/500—1/1200. The hemolytic system included equal volumes of hemolytic serum, diluted according to the titre, and a 5 per cent solution of sheep erythrocytes. Dry guinea pig serum was used as complement. The CFT results were read after 30 minutes, again on adding the hemolytic system, and again 35 hours after setting up the reaction.

Results of Investigation. Out of 30 experimental animals, 5 succumbed to shock following 2—3 casein injections. The remaining 25 rabbits depending upon the dates of examination were classified into the following 5 groups.

Experimental groups	Dates of examination	Number of animals
1	1—2 weeks (6—16 days)	8
2	3—4 weeks (18—33 days)	6
3	6 weeks (41—44 days)	7
4	7 weeks (50—54 days)	2
5	2.5 months (80 days)	2
	A total of	25

I. group. In 1—2 weeks after commencement of the experiment the rabbits developed a mild proteinuria (0.066‰ to 0.132‰) as well as marked shifts in the protein blood fractions (see Table 1). Initially, these were, manifested by an appreciable fall in the albumin content, a definite rise in the α_1- and α_2-globulin levels, and a sharp increase in the β-globulin level. No marked changes of

Table 1. *Protein, blood serum glycoprotein and protein level in the urine*

NN of animals	Duration of experimental days	Experimental group	Uroprotein in ‰	Total blood serum protein % %	Blood serum protein fraction content in per cent to the total protein					Blood serum glycoprotein content in per cent to the total glycoprotein level				
					Albumins	Globulins				Albumins	Globulins			
						α_1	α_2	β	γ		α_1	α_2	β	γ
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Normal	—	—	—	7.2±0.37	56±3.67	6±2.01	10±1.6	13±2.33	15±2.33	15±1.86	19±3.32	23±2.21	24±2.21	18±2.12
12	13	I	0.132	7.2	43	8	13	22	14 (7+7)	16	20	23	24	17 (12+5)
14	16	I	0.132	6.9	35	10	12	27	16 (7+9)	16	17	23	24	20 (10+10)
17	13	I	0.066	7.2	43	8	13	24	12	13	18	28	26	13
18	13	I	0.132	6.55	32	5	16	20	18 (8+10)	15	20	26	26	13 (8+5)
15	10	I	unditerm.	6.5o	47	8	13	18	17	14	20	22	26	18
1	18	II	0.264	6.98	41	8	17	23	11 (8+3)	12	16	25	32	15 (9+6)
2	22	II	unditerm.	6.55	41	8	17	25	10 (5+5)	14	18	25	28	15 (11+4)
3	23	II	0.264	6.12	45	7	14	18	16	16	20	23	24	17
13	23	II	0.568	5.9	37	9	13	26	15	12	19	21	29	19
4	33	II	unditerm.	6.55	32	5	13	22	28	13	16	24	30	17
20	41	III	0.528	4.81	41	7	12	23	17 (10+7)	11	18	23	29	19 (12+7)
22	42	III	0.528	4.81	45	8	11	22	14 (10+4)	12	19	24	28	21 (15+6)
24	42	III	0.264	6.9	53	10	9	13	15	14	23	23	25	15
26	42	III	0.528	5.9	44	7	8	22	19	13	19	25	26	17
28	42	III	1.056	4.8	47	6	10	21	16	15	17	24	30	14 (8+6)
5	50	IV	1.056	4.38	37	8	13	28	14 (7+7)	12	17	25	33	13 (7+6)
9	54	IV	4.004	5.8	47	7	9	21	16 (6+10)	14	17	22	29	18 (10+8)
6	80	V	0.528	5.9	46	6	11	16	19 (7+12)	14	20	23	25	18 (11+7)
8	80	V	0.528	5.9	46	9	11	17	17 (7+10)	15	18	23	24	20 (10+10)

the glycoprotein content could be revealed. Of interest was the appearance of two constituents in the γ-globulin fraction, the same division being observed also in the γ-glycoprotein fraction.

In animals of this group macroscopic and histological alterations were found in the spleen and lymph nodes.

The spleen was somewhat enlarged, flaccid and presented distinct follicles. In the centre of the latter and particularly at their periphery in the marginal zones, many large cells of the reticular and plasmatic types were encountered. Most of them were distinguished by a pronounced pyroninophilia (Fig. 1) and

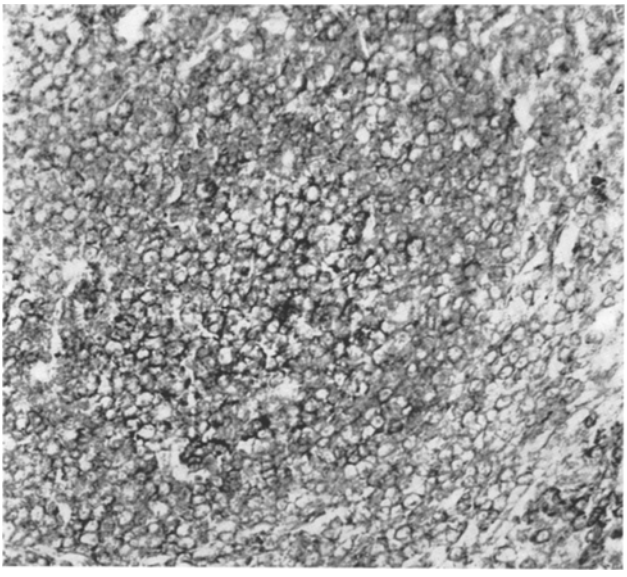

Fig. 1. Reticulo-plasmacytic transformation of the elements in the splenic follicles. Brachet reaktion. × 40

only a few of the cells contained in their protoplasm PAS-positive granules (PAS-positive cells). In addition to this, delicate PAS- and fibrin-positive reticular structures were discovered pericellularly in the zone of the reticulo-plasmacytic hyperplasia. The red pulp contained much blood.

Lymph nodes, which were considerably enlarged, presented the same picture of reticulo-plasmacytic hyperplasia of their follicles, but this was not so distinctly accentuated when compared with the spleen. The CFT involving proteins isolated from the spleen, kidneys and liver, on the one hand, and blood serum of the same animals, on the other, proved but slightly positive (see Table 2).

II. group. During the subsequent 2—3 weeks of the experiment, along with a further rise of the β-globulin and α_2-globulin levels, a sharp increase in the β-glycoproteins became apparent (see Table 1). This was paralleled by an augmenting proteinuria and a decline of total blood proteins.

In this experimental group macro- and microscopic changes were found in the spleen, lymph nodes, kidneys and the liver.

The spleen increased in weight up to 4.0—5.0 g (in control animals it weighed 1.5—2.5 g), it was flaccid and its section presented large follicles. The

perifollicular zones of the reticuloplasmacytic hyperplasia became quite extensive, and in individual follicles they almost merged with their light-coloured centres. The number of plasmatic cells was increased, with individual giant polykaryocytes making their appearance here and there. The amount of RNA in the reticular and plasmatic cells was also increased. There was, furthermore, a considerable rise in the number of PAS-positive cells (PAS-reaction disappeared following

Table 2 *Comparative characteristics of morphological and immunological data*

NN of rabbits	Group of experiment	Day of experiment	Degree of organ affection			Results of complement fixation test 4		
			Spleen[1]	Kidneys[2]	Liver[3]	Spleen	Kidneys	Liver
12	I	13	0	0	—	++	++	—
17	I	13	0	0	—	+	++	—
18	I	13	0	0	—	+	+	—
1	II	18	000	00	0	+++	+++	—
2	II	22	00	00	0	+	++	—
3	II	23	00	00	—	++	++	—
13	II	23	00	00	—	++	+++	—
20	III	41	000	000	00	+++	++	+++
5	IV	50	00	0000	00	++	+++	+++
9	IV	54	0000	0000	0	+++	+++	+
6	V	80	000	000	00	++	+++	++
8	V	80	000	000	00	++	+++	++

1. *Splenic Lesion.*

0— pre-amyloid stage
00— small amounts of amyloid in the periphery of follicles
000— replacement of follicles by amyloid and small amounts of the latter in the red pulp
0000— replacement of follicles and of the red pulp by amyloid.

2. *Renal Lesion.*

0— pre-amyloid stage
00— amyloid in pyramids
000— amyloid in pyramids and isolated glomerules
0000— amyloid in pyramids and numerous glomerules.

3. *Hepatic Lesion.*

0— pre-amyloid stage
00— small amounts of amyloid along the periphery of lobules.

4. *Complement Fixation Test.*

+++ accentuated delay with traces of hemolysis
++ partial delay with traces of hemolysis
+ manifest hemolysis with traces of non-dissociated erythrocytes
— complete hemolysis.

incubation with diastase). The protoplasm of the markedly pyroninophilic PAS-positive cells, when stained with Congo red, presented minute brick-red granules, whereas the Feulgen's reaction disclosed chromatin grains. In this instance the nuclei had a poorly stained appearance. In individual sections groups of these cells were surrounded by accumulations of a finely fibrillar PAS-positive material which gave a light-brick colour when stained with Congo red, whereas picrofuchsin stained yellow. With toluidine blue, it gave an orthochromatic coulor and in the polarisation field the material was middly anisotropic. Apart from this, giant polykaryocytes which by their tinctorial properties in no way differed from

the RNA-rich and PAS-positive follicular cells, were revealed in the red pulp, characterised by much blood and by lymphostasis.

In 4 rabbits out of 6 in this group an amyloid substance was discovered within the marginal zone of the follicles on the 18—22 experimental day. Within this substance pyroninophilic and PAS-positive cells were immured (Fig. 2). The amyloid masses stained well with Congo red, were metachromatic with methyl-violet and orthochromatic in respect to toluidine blue, distinctly PAS-positive (incubation with diastase led to only a slight weakening of the reaction), and had pyroninophilic properties (incubation with ribonuclease did not result in complete disappearance of pyroninophilia). When stained with picrofuchsin, the amyloid

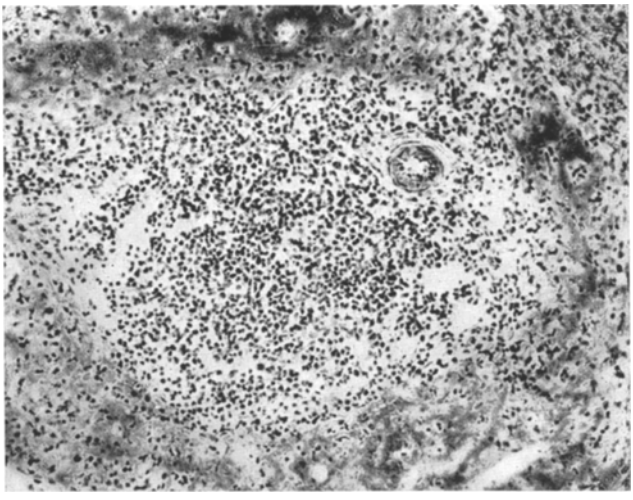

Fig. 2. Amyloid mass in the marginal zone of the splenic follicle. Congo red. × 35

assumed an olive colour, whereas Mallory's method rendered it lightblue. In a polarisation microscope it produced a marked anisotropic effect, and exhibited a typical finely fibrillar and reticular structure.

The lymph nodes were 1.5—2 times their normal size. As compared with the first group, here the reticuloplasmacytic hyperplasia of the follicles appeared to be more pronounced with more abundant RNA in the cells. The number of PAS-positive cells was insignificant.

The kidneys were somewhat enlarged and flaccid. As a rule, small amounts of the amyloid substance were found in the interstitial spaces of the pyramidal papillae and, on much rarer occasions, in the stroma along the course of the straight tubules and vessels. Glomeruli were enlarged, their capillary membranes were thicker than usual, markedly PAS positive, with individual cells rich in RNA. No amyloid was found in the glomeruli.

The liver was somewhat enlarged, flaccid and congested. Perivascularly, more often at the periphery of the lobules, one could see accumulations of cells belonging to the lymphoreticular-plasmacytic series, with distinct pyroninophilia of their protoplasm.

At these periods of the experiment the results of the CFT appeared more clear-cut, particularly with respect to proteins in the kidneys and spleen (see Table 2).

III. group. Characteristic for the animals in this group was a substantial drop of level of the total blood protein (down to 4.8 per cent), accompanied by a further rise of proteinuria (up to 1.056‰). The β-globulin and β-glycoprotein components continued to be high, whereas the α_2-globulin level returned to normal values (see Table 1). As in the preceding two groups, some of the animals showed a division of the γ-globulin fraction into two sub-fractions. A similar division also took place in the instance of the γ-glycoproteins, whereby the bulk of the PAS-positive substance was part of the fast-migrating constituent.

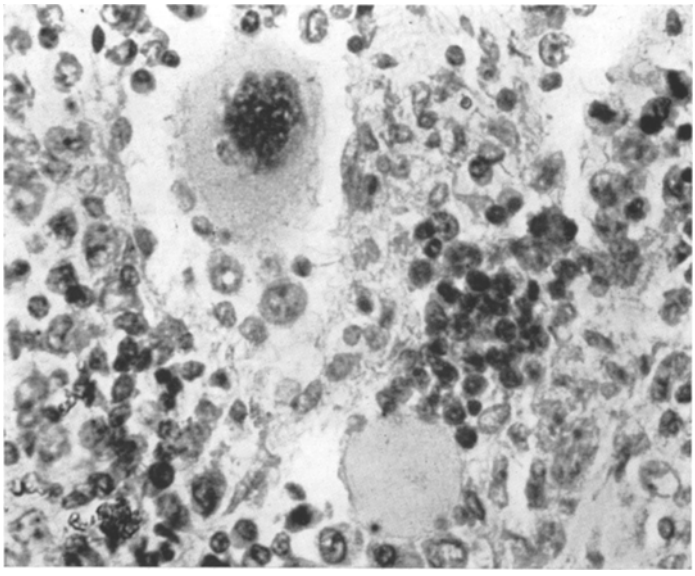

Fig. 3. The splenic red pulp giant cell protoplasm is sharply PAS-positive. PAS-reaction. × 250

On pathomorphological examination, changes were revealed in the same organs as in the second group, but they were more prominent in the spleen and kidneys.

Amyloidosis of the spleen was found in 5 out of 6 animals in this group III. The weight of the spleen reached 6.0—8.0 g; it was indurated. There was an increase in the amyloid substance at the periphery of follicles, although the number of pyroninophilic and PAS-positive cells immured within the amyloid deposits decreased. In the congested red pulp, which disclosed lymphostasis, there were numerous giant polykaryocytes (Fig. 3). The protoplasm of these cells was rich in RNA, in granules of PAS-, and in Congo red-positive material. Appropriate enzymes failed to eliminate the pyroninophilia and PAS-reaction of some of the giant cells. It was possible to see the formation of small deposits of amyloid was at the site of these cells.

In renal pyramids there was an increase of amyloid deposits; small clumps of amyloid appeared as well along the straight vessels and tubules in the cortico-medullary zone. Small amounts of the amyloid substance also were found in isolated glomeruli along capillary membranes.

In the liver of 5 animals the changes did not differ from those described for the second group, but were more marked. In only one rabbit (No. 20) was amyloid

revealed in the liver on the 41st day of the experiment. Amyloid deposits lay perivascularly at the periphery of lobules and, in places, were surrounded by agglomerations of reticulo-plasmacytic pyroninophilic elements.

In the lymph nodes the alterations were similar to those tabulated for the animals of the second group. The CFT proved to be distinctly positive, not only when performed with splenic and renal proteins, but also when liver proteins served as an antigen (see Table 2).

IV. group. After 1.5 months the proteinuria reached its peak value (4.004‰). The electrophoretic picture of the blood serum was in no way different from

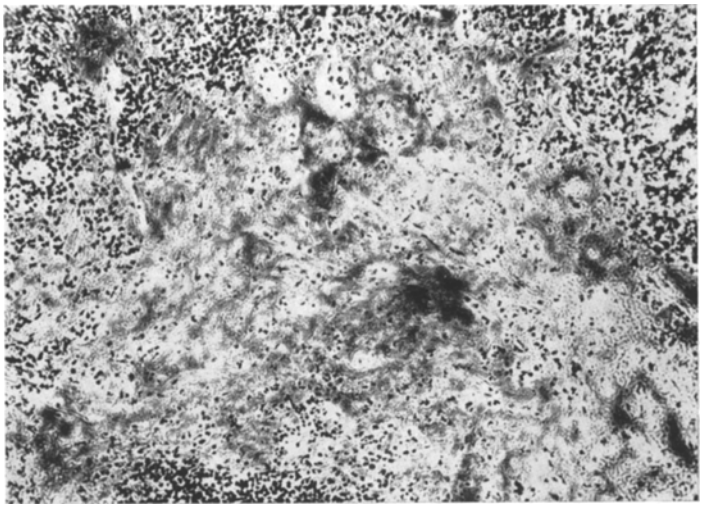

Fig. 4. Amyloid in the red pulp of the spleen. Congo red. × 145

that seen in the preceding experimental group, except that the levels of α_1- and α_2-globulins dropped to their normal values (see Table 1).

Morphologically, renal changes distinguished this group from the previous one.

In the kidneys, in addition to the amyloid substance in pyramids and in the corticomedullary zone, amyloid deposits were also found in many glomeruli along the capillary basement membranes, which led to a vacancy of individual loops.

The CFT proved distinctly positive when carried out with proteins coming from those organs where amyloidosis was particularly evident (see Table 2).

V. group. After 80 days of experimentation the electrophoretic changes in the proteins and glycoproteins largely levelled out, although the total protein and albumin levels still continued to be rather low, whereas the γ-globulin fraction presented two constituents (see Table 1).

In the animals of this group amyloid deposits were found in the spleen, kidneys and the liver. The lesions were most pronounced in the spleen, both in its follicles and in the red pulp (Fig. 4), and in the kidneys, where amyloid was seen in the cortex and in the pyramids.

Late in the course of the experiment the CFT proved positive with proteins of the spleen, kydneys and the liver, but the most clear-cut result was obtained with renal proteins (see also Table 2).

Discussion of Results

When confronted with the results of the experiment obtained at different periods of time, it is possible to note definite relationships in the dynamics of the biochemical, morphological and immunological changes.

Early in the experiment (in the first two weeks), biochemical alterations concern serum proteins, and in the main, imply a sharp rise of the β-globulin fraction, with only an insignificant increase in the α_2-globulins and a drop in the level of albumin. Such changes of serum proteins are typical for an early stage of experimental amyloidosis (Giles, Calkins 1958, Richter 1956, Christensen, Rask-Nielsen 1962).

In the opinion of some authors these changes represent an indispensable form of "dysglobulinemia" in the development of experimental amyloidosis (Huestis, Jaeger 1960).

At the same time in the experiment, a marked reticulo-plasmacytic hyperplasia of the lymphoid tissue becomes apparent in the lymph nodes, and particularly in the spleen within the peripheral area of the follicles. This is accompanied by a sharp increase of the RNA content in the reticular and plasmatic cells. It is a well known fact, that at the time of their formation immunological processes take part, first and foremost, in the spleen and lymph nodes, here one may see an increase in the number of RNA rich plasmatic cells (Rapoport 1957, Fagraeus 1958), which play so important a role in the biosynthesis of proteins and in the formation of antibodies (Wuhrmann, Wunderly 1952). It is only natural to expect a low titer of antiorgan antibodies at that time marking the formation of immunological processes. It is just this fact that explains the absence of clear-cut positive results of the CFT at early periods in the experiment.

After 3—4 weeks from the onset of the experiment, apart from the already noted shifts in the protein level, a sharp increase of β-glycoproteins can be seen in the blood serum. A rise of the γ-globulin fraction, considered as characteristic of experimental amyloidosis (Letterer 1934, 1939, Teilum 1954), occurs only in individual cases. Much more frequently one may see the second constituent appearing in the γ-globulin and γ-glycoprotein fractions even at early periods of the experiment. A comparison of both electrophoregrams (for proteins and glycoproteins), demonstrates the prominence of the PAS-positive substance which corresponds to the fast-moving component.

It should be noted that changes in the relations of serum proteins and glycoproteins occurring during the formation of the amyloid were recorded not only in the animals of different groups, but in the very same animal as well. Hence, at these early periods of the experiment, anomalous proteins and glycoproteins make their appearance in the blood serum, and may be regarded as compounds typical of a pre-amyloid stage (Huestis, Jaeger 1960, Richter 1956).

At the same time in the experiment a further rise of plasmacytic hyperplasia and an augmented RNA content in plasmatic cells may be observed to appear in the reticulo-endothelial system, and especially in the spleen. Worthy of note is the fact, that at early periods of the experiment only isolated cells of the reticulo-plasmacytic series give a prominent PAS-reaction. At the time marked by the vise in blood serum β-glycoprotein, however, the number of such cells increases an appreciable manner with the appearance of Congo red positive granules

within them. Typical also are alterations of the nuclear chromatin in these cells, with the chromatin grains entering the plasma. At the same time in the experiment pericellular agglomerations of a finely fibrillar material can be observed, which is likely to give typical staining reactions for amyloid, and an anisotropic effect in the polarisation field.

These changes in the reticulo-endothelium, which are in full accord with the data furnished by TEILUM (1954—1961), COHEN, CALKINS and LEVENE (1959), represent a morphological manifestation of the pre-amyloid stage. And in fact, at this stage, 18—20 days after the commencement of the experiment, one half of the rabbits were seen to develop amyloidosis of the spleen.

In this respect, it is characteristic for amyloid to become deposited at the periphery of follicles, within the area of the maximal plasma content, amidst cells rich in nucleic acids and polysaccharides.

It should be stressed that these cellular alterations, characterizing the pre-amyloid stage, are factors causing the development of the auto-immunisation state. Thus, an increased amount of plasma in the cells of the reticulo-endothelium and the rise of the RNA level therein testify to an intensified formation of antibodies (FAGRAEUS 1958). Our immunological data confirm this proposition; on the other hand, however, they prove these antibodies are produced against pervertedly functioning cells of the reticulo-endothelium. In this series of experiments the positive results of the CFT appeared to be most clear-cut for the proteins of the spleen and kidney, where amyloid substance may be recognized morphologically.

Mention is to be made of the fact that amyloid deposits in kidneys at these periods are seen only in the papillae and stroma of the pyramids, and that their degree is directly related to the extent of proteinuria. This gives ground to suppose that the initial stage of the renal amyloidosis is linked with transudation of paraproteins into the renal tissue and with resorptive insufficiency of the renal lymphatic system (RANDERATH 1947).

Late in the course of the experiment (1.5—2.5 months) amyloidosis begins to develop in the spleen, kidneys and in the liver. In the glomeruli of the kidneys, in the liver, as well as in the spleen, the formation of the amyloid substance is preceded by the appearance of pyroninophilic and PAS-positive cells of the reticulo-endothelium. This is also mentioned by TEILUM (1956). At this time in the spleen, at the periphery of follicles, but mostly in the red pulp, one may see the appearance of numerous giant polykaryocytes at the sites where it is possible to trace the formation of the amyloid substance. Like amyloid, the protoplasm of the giant macrophages gives positive pyroninophilic and PAS-reactions which could not be eliminated by corresponding enzymes. This information supports the opinion expressed by TEILUM (1957), who attributes the appearance of giant cells in experimental amyloidosis, not to the amyloid resorption as is believed by many workers, but to the production of the amyloid substance, at the background of which lies tissue dysproteinosis.

The formation of amyloid in an area marked by a pronounced plasmacytosis with cellular accumulations of nucleoproteins and polysaccharides, just as at the site of giant macrophages, which are endowed with a high functional activity in the course of immunogenesis (POKROVSKAYA et al. 1959) allows one to consider

amyloid to be in large measure a product of cellular transformations, the nature of which must be attributed to autoimmunological phenomena. However, apart from the cellular substrate, amyloid also includes plasmatic proteins. This is born out by the data derived from the biochemical investigations of the animals' blood serum and amyloid. Our investigations of the blood protein level, likewise, have shown that with pronounced amyloidosis of the organs, irrespective of the extent of proteinuria, there occurs a sharp decline primarily of α_2- and β-globulin content, with the retention of pathological deviations in the γ-globulin value, i.e. in those protein fractions, which are part of the amyloid substance (LARSEN 1957, WAGNER 1955). Late in the experiment the CFT was, as a rule, distinctly positive in those organs affected mostly by amyloidosis. The CFT data make it possible to believe that, as pointed out by a number of authors (PARNES 1956, 1957, VAZQUEZ, DIXON and NEIL 1957, VOGT, KOCHAM 1960) amyloid has a definitely specific function as an autoantigen, this explaining the rise of antiorgan antibodies at this time of the experiment.

In this connection a question arises: to what extent are these experimental data useful in explaining the pathogenesis of secondary amyloidosis in man? It seems to us that in answering this question the detection of specific antibodies in the blood of patients affected with amyloidosis may be of some help. With this purpose in view experiments were staged, involving the same complement fixation test (CFT) as one of the most sensitive serological reactions.

For immunological investigations blood serum was used from the cadavers of 9 patients, who had died of chronic ulcerative pulmonary tuberculosis complicated by amyloidosis.

Blood taken from healthy subjects served as controls, as well as blood serum obtained from cases of pulmonary tuberculosis with no signs of amyloidosis. To inquire into the effect of post mortem changes on the CFT, control experiments were staged, with the blood being taken from cadavers from cases of coronary insufficiency.

As antigens, protein fractions of amyloid were employed, isolated according to HASS and SCHULZ (1940) from the amyloid-degenerated liver and kidneys, taken from the same cadavers that served as a source for the blood. One of these fractions — the basic amyloid one — was precipitated with acetic acid from the tissue alkaline extracts. Their electrophoretic migration rate showed it included two components close to the blood serum α_1- and γ-globulins, both components gave the PAS-positive reaction. The second, water soluble fraction was precipitated with alcohol and its electrophoretic migration rate was close to that of the serum β-globulin. Its smear produced weak metachromasia with toluidine blue. Both antigens were propared in dilutions of 1/50, 1/100, 1/300, 1/500, 1/1000, 1/1200, 1/1500.

Since the water-soluble fraction, as it was found out at a later date, was apt to produce a hemolytic effect in all dilutions, the basic, amyloid fraction alone was employed in all the experiments.

The CFT was staged by following the pattern described earlier in this paper. The sera examined were prepared in dilutions of 1/50, 1/500, 1/1000, 1/1200, 1/1500.

Nine blood sera were examined and about one hundred complement fixation tests made. The final results of the investigation are listed in Table 3 (this in-

Table 3. *Complement fixation test involving amyloid proteins and various blood sera*[1]

Serum under investigation	Dilution of antigen and serum	Hepatic amyloid			Renal amyloid		
		VII	VIII	IX	VII	VIII	IX
I. Blood serum in amyloidosis	1/500		+++			+++	
	1/1000	+++	+++	+++	++++	++	+++
	1/1200	++++	++++	++++	+++	++	++++
	1/1500	+++			+++		
II . Blodd serum of healthy subjects	1/500		+				
	1/1000	—	—	—	+	+	—
	1/1200	—	—	—	+	—	—
	1/1500	—			—		
III. Cadaveric blood serum in cases of tuberculosis	1/1200	—	+	—	—	—	—
IV. Cadaveric blood serum in cases of coronary insufficiency	1/1200	—	—	—	+	—	—

[1] VII—IX numbers of cases; ++++ complete delay of hemolysis; +++ delay with traces of hemolysis; ++ partial delay with distinctly pronounced hemolysis; + manifest hemolysis with traces of non-dissociated erythrocytes; — complete hemolysis.

cludes maximal sera and antigen dilutions with which the test results were still sufficiently legible).

Positive complement fixation reactions involving proteins of amyloid-degenerated organs and the proteins of the blood serum of the same patient give sufficient ground to consider that in the pathogenesis of amyloidosis in man of great importance are auto-aggressive processes, with the proteins of the body acting as antigens. A point in favor of this is the similarity between the chemical composition of amyloid and of certain pathological proteins appearing in the blood during amyloidosis.

Summary

The information submitted makes it possible to speak of the participation of immunological reactions in the pathogenesis of amyloidosis, which, in all probability, come about by a mechanism of auto-aggression. Furthermore, two stages may be distinguished in the development of amyloidosis:

First stage, the formation of auto-antigens and of corresponding auto-antibodies, this appearing as a result of a deranged protein-synthetizing function of the reticulo-endothelium.

Second stage, association of auto-antigens with auto-antibodies followed by the formation of specific complexes and their fixation in tissues in the form of an amyloid substance.

Über die Pathogenese der Amyloidose

Zusammenfassung

Auf Grund von Untersuchungen über experimentelle Amyloiderzeugung mit Kasein wird die Amyloidose als Autoimmunkrankheit gedeutet, die in zwei Phasen abläuft. In der ersten Phase erfolgt die Bildung von körpereigenen Antigenen

und entsprechenden Antikörpern. Die Antigene gehen aus einer veränderten Proteinsynthese durch das retikuloendotheliale Gewebe hervor. In der zweiten Phase führt die Antigen-Antikörperreaktion zur Bildung spezifischer Verbindungen mit geweblicher Fixation derselben in Form von Amyloid.

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