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Histochemical Study on Systemic Amyloid Microdeposits with Special Reference to Parathyroid Intrafollicular Deposits

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Summary. This study was carried out in order to define the histochemical behaviour of the substance present in follicles of normal parathyroid glands obtained at routine autopsies. It was noticed that these follicles with typical amyloid staining properties are constantly present in old persons; furthermore the amyloid in question presents histochemical characteristics which identify it as Apudamyloid.

With this in mind we studied the possible, concomitant, systemic, amyloid microdeposits; we noticed that intrafollicular Apudamyloid of the parathyroid gland is often encountered together with immune deposits which are sometimes present in the same gland though not necessarily in the follicles.

Key words: Ageing — Amyloidosis — Parathyroid glands.

Introduction

The encounter of staining properties typical of amyloid in the substance contained in parathyroid follicles is a recent discovery which has not yet been fully defined. This is also due to the fact that the problem was mainly studied in pathological glands obtained at biopsy (Leedham and Pollock, 1970). Only recently was the presence of a substance with histochemical properties typical of amyloid also encountered in normal glands (Lieberman and De Lellis, 1973; Anderson and Ewen, 1974; Hansson and Nilsson, 1974).

In the light of these recent findings we decided to study parathyroid glands obtained at routine autopsies; we dealt with the problem by including it in the wider field of studies on systemic amyloid microdeposits (Ravid *et al.*, 1967).

We were particularly interested in the typing of this intrafollicular amyloid according to the scheme of Pearse *et al.* (1972) who differentiates two kinds: apudamyloid and immunamyloid.

Material and Methods

The study was performed on parathyroid glands obtained at routine autopsies. We chose our material by simply eliminating all cases with conditions of autolysis, including those at an early stage. Although more than one parathyroid gland was taken for each case, with the aim of standardizing the study, only one was studied for each subject.

Data concerning the 13 cases studied are presented in Table 1.

Table 1

Case number	Autopsy number	Sex	Age	Pathological chart
1	1121/74	F.	66	Bilateral bronchial pneumonia; generalised arteriosclerosis; chronic pancreatitis
2	1124/74	M.	73	Massive pulmonary thromboembolia
3	1143/74	M.	65	Haemorrhage from rhexis of oesophageal varices in carcinoma in cirrhotic liver. Neoplastic thrombosis of the portal vein
4	1149/74	M.	83	Metastasizing squamous carcinoma of the lung
5	1153/74	F.	86	Bronchial pneumonia; generalized arteriosclerosis
6	1163/74	M.	61	Haemorrhage from rhexis of oesophageal varices in splenomegalic liver cirrhosis
7	1168/74	F.	90	Carotid thrombosis with extensive cerebral softening
8	1174/74	M.	83	Adenocarcinoma of the colon with metastasis
9	1186/74	F.	74	Massive pulmonary thromboembolia in thrombosis of iliac veins
10	1197/74	F.	86	Adenocarcinoma of the pancreas head with infiltration to the duodenum. Generalized icterus
11	1218/74	F.	78	Stomach adenocarcinoma. Bilateral bronchial pneumonia
12	1233/74	F.	74	Reticulum cell sarcoma infiltration in the left hemithorax, (pleural, pulmonary, costal, muscular)
13	1302/74	M.	75	Generalized primitive amyloidosis (cardiovascular). Bronchial pneumonia

The parathyroid glands, fixed in 10% neutral formalin immediately after autopsy, were then embedded in paraffin. 5 μ thick sections from each block were removed and then stained with the following:

1) Hematoxylin-eosin.
2) Periodic acid-Schiff (PAS).
3) Congo-red in alcaline alcoholic solution according to Puchtler *et al.* (1962).
4) Thioflavin T according to Vassar and Culling (1959).
5) Toluidine blue from pH 1 to pH 4.
6) Staining for tryptophan with dimethylaminobenzaldehyde (DMAB).

The Congo-red stainings were viewed in polarized light; in each case, a blank section, carefully dewaxed, was viewed in a fluorescent microscope (Leitz Orthoplan fitted out with "Fluoropak" vertical illuminator according to Ploem, with selector in position 1 corresponding to the U.V. band).

The staining for tryptophan was carried out on a series of slides decolorized with a 3% HCl solution in ethylic alcohol; these slides had been previously stained with Congo-red and the co-ordinates of the birefringent and dichroic follicles had been determined in polarized light (by means of suitable mechanical stage equipped with graded specimen guide). This was done to test reaction of the substance contained in the above-mentioned follicles to the staining for tryptophan without erroneously considering other non-birefringent and dichroic follicles.

All in all the co-ordinates of 196 follicles out of a total of 12 cases were determined; in one case (no. 11) this was not possible due to the enormous amount of follicles present and positive for demonstration of amyloid.

Table 2

Reaction or stain	Immunamyloid	Apudamyloid	Amyloid in Parathyroid glands
PAS reaction	+	+	+
Congo red—Dichroism	+	+	+
Thioflavin T	+	+	+
Metachromasia Toluidine blue pH 4.0			+
Tryptophan (DMAB)	+++	−	−(+)
Autofluorescence (U.V. light)	+	−	−(+)

Comparison of the histochemical characteristics of Immunamyloid and Apudamyloid (Pearse *et al.*, 1972) and behaviour to the same reactions of the Amyloid detected in the Parathyroid glands.
−(+): indicates a practically negative reaction.

Furthermore a systematic research was carried out on all the cases with the aim of pointing out amyloid microdeposits in organs examined at autopsy. We took into particular account the following;—encephalon, lung, heart, liver, kidney, pancreas, hypophysis, epiphysis, thyroid, prostate. Two Congo-red stained histological sections (non consecutive) of these organs were viewed in polarized light. In cases with amyloid, the sections were also decolorized and restained with the DMAB method for tryptophan thus assessing its presence or absence in these deposits.

Results

During the various stages of the research 4 sections (non consecutive) of each parathyroid gland were stained with Congo-red; in at least one for each case, one or more birefringent and dichroic follicles were seen in polarized light. On the basis of these data we can say that the presence of follicles containing a substance with staining properties typical of amyloid is constantly encountered in normal parathyroid glands of old persons.

The histochemical reactions were selected on the basis of the study carried out by Pearse *et al.* (1972). This author, in fact, distinguishes two amyloid kinds: a kind said immunamyloid and another said apudamyloid.

Such a distinction puts on the tryptophan discriminant presence in the first amyloid kind only. Therefore, this one will stain blue by means of the DMAB reaction for the above named aminoacid, while apudamyloid will remain unstained.

Moreover the tryptophan becomes autofluorescent when irradiated with U.V. light. Hence the immunamyloid deposits exhibit a bright blue autofluorescence when observed by means of a fluorescence microscope.

Concerning the other reactions (PAS, Congo red, Thioflavin T), these give the same results in both amyloid kinds considered, not helping hence to distinguish them.

The results obtained from the study on parathyroid sections stained with the various previously mentioned methods are presented in Table 2.

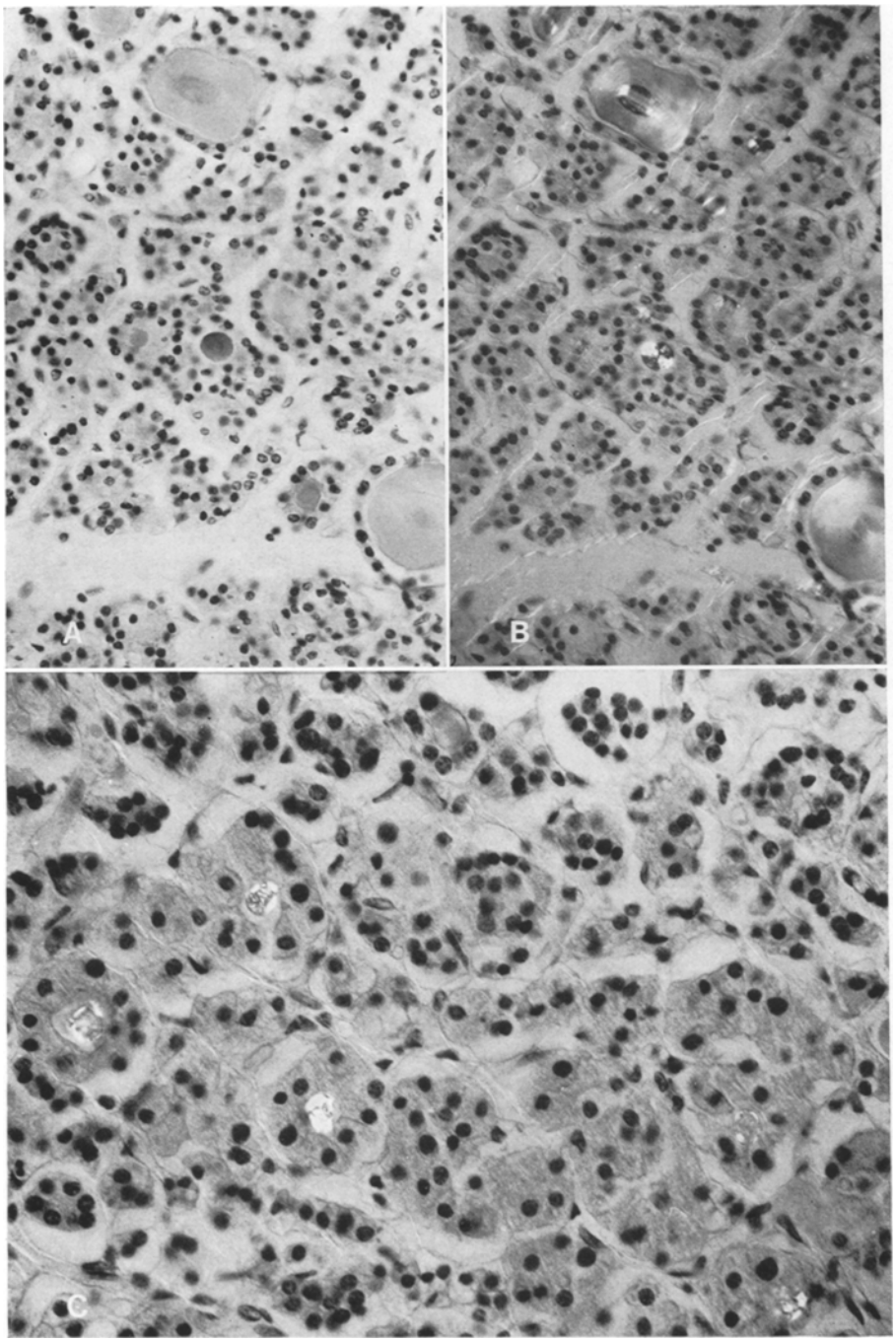

Fig. 1. (A) Aut. 1218/74 (Case No. 11); intrafollicular amyloid deposits in parathyroid glands. (Congo red ×100). (B) Same as in previous figure viewed in polarizing light. (C) Same case: intrafollicular amyloid deposits. Unlike Fig. 1 (A) where the follicles were lined by chief cells, here the follicles are made up of oxyphil cells. (Congo red ×100)

Table 3. Table summarizing the behaviour to the Congo-red and DMAB reactions of the amyloid microdeposits in various organs considered. The meaning of the result −(+) obtained for the DMAB reaction in Parathyroid glands is "practically negative" and is referred to the intrafollicular secretion only

Cases	Epiphysis		Hypophysis		Thyroid		Parathyroid		Prostate		Brain		Heart		Lung		Liver		Kidney		Pancreas	
Number	C.r.	Trp.	C.r.	Trp.	C.r.	Trp.	C.r.	Trp.	C.r.	Trp.	C.r.	Trp.	C.r.	Trp.	C.r.	Trp.	C.r.	Trp.	C.r.	Trp.	C.r.	Trp.
1	+	−	+	−	−		+	−(+)	−		−		−		−		−		−		−	
2							+	−(+)					+	+	+	+	−		−			
3					+	−	+	−(+)					−		−		−		−		+	−
4			+	−	−		+	−(+)			+	−	−		+	+	−		−		−	
5			+	−	−		+	−(+)			−								−		+	−
6			+	−	−		+	−(+)	+	±	−		−		−		−		−		+	−
7	−				−		+	−(+)			+	−	+	+	+	+	−		−		+	−
8					−		+	−(+)	+	±	+	−	−		−		−		−		−	
9					−		+	−(+)	−		−		−		−		−		−		−	
10					−		+	−(+)			−		+	+	−		−		−		+	−
11	−		+	−	−		+	−(+)	−		+	+	−		−		−		−		−	
12	+	−			−		+	−(+)			−		−		−		−		−		−	
13					+	+	+	−(+)	+	±			+	+	+	+	+	+	+	+	+	+[a]

C.r. = Congo-red.
Trp. = Tryptophan.
[a] Vasal amyloidosis.

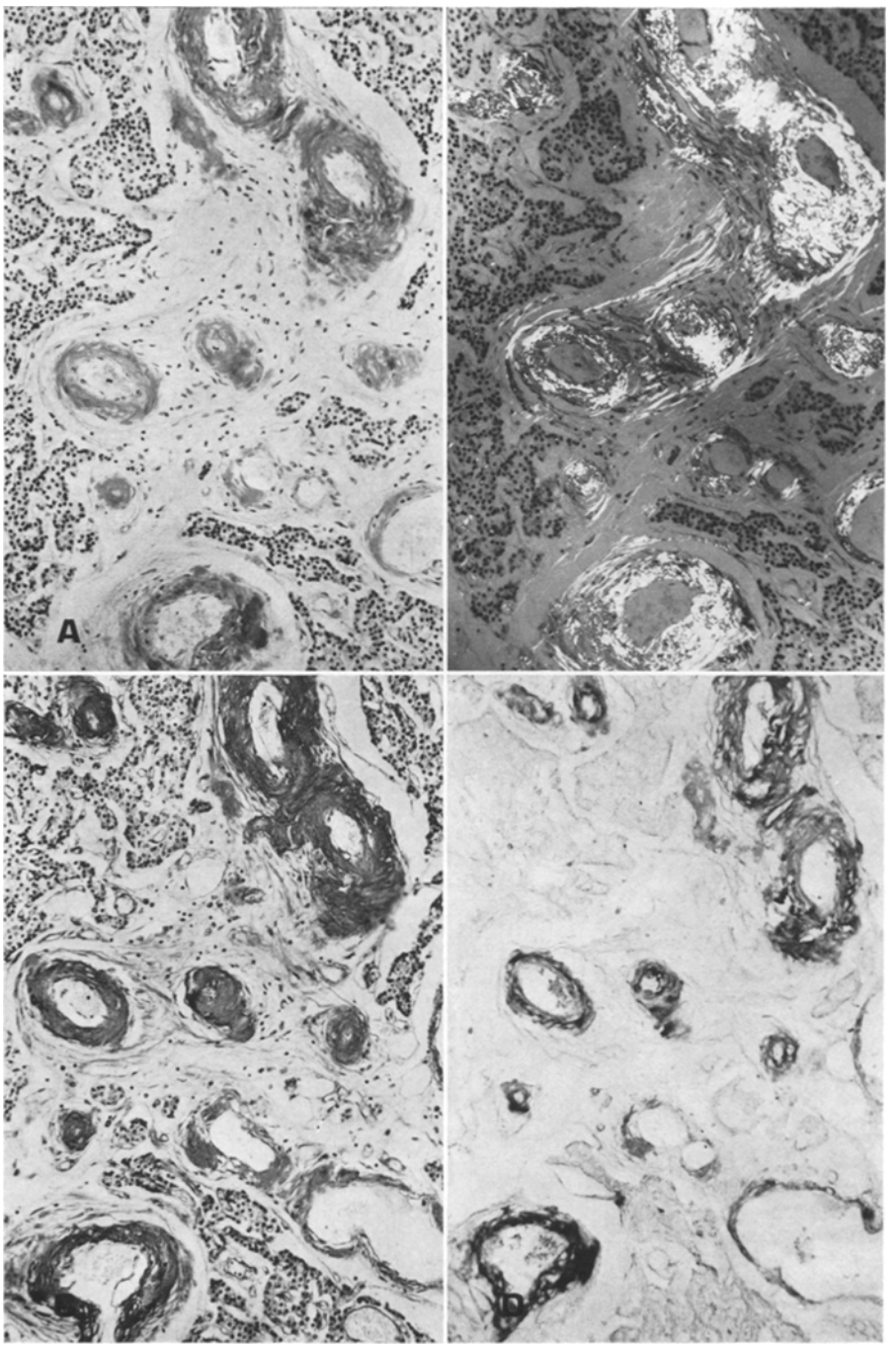

Fig. 2. (A) Aut. 1302/74 (Case no. 13); vasal amyloidosis in parathyroid gland. (Congo red ×40). (B), (C), (D): Same as previous figure viewed respectively in polarized light, with PAS reaction, with DMAB method for tryptophan

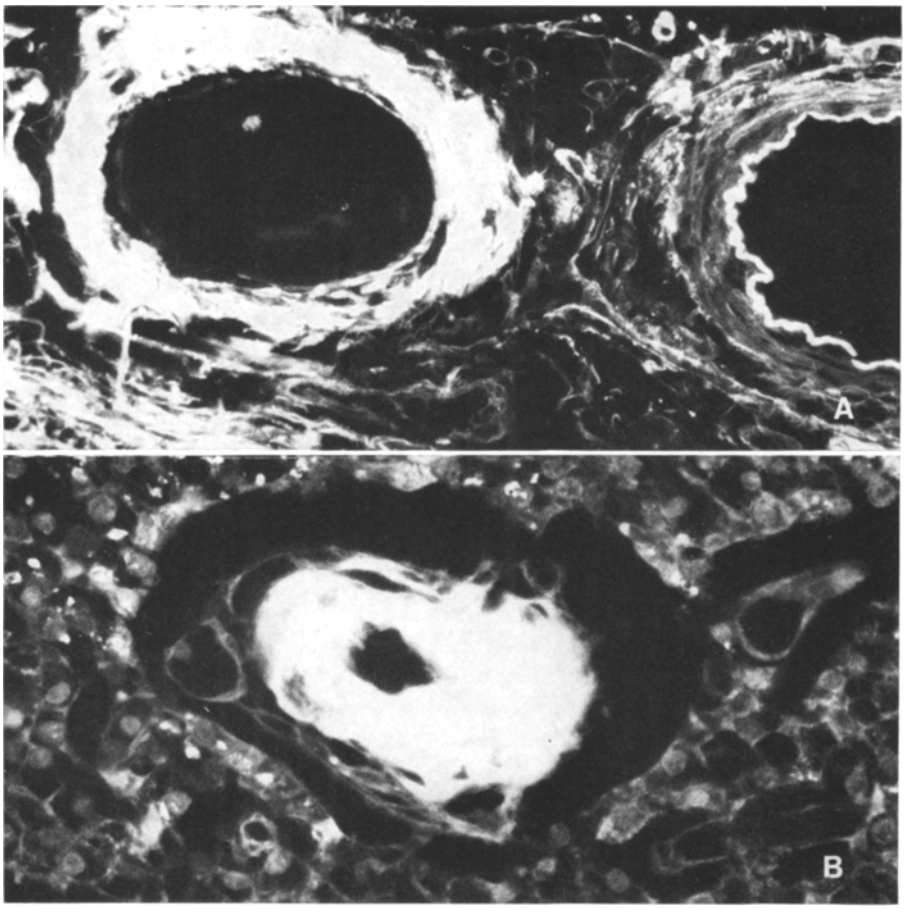

Fig. 3. (A) Aut. 1302/74 (Case no. 13); parathyroid vessel infiltrated by amyloid (left) compared with a normal one of the same gland. Amyloid results autofluorescent in U.V. light. (×40). (B) Parathyroid vessel infiltrated by amyloid autofluorescent in U.V. light. (×100)

In addition to what is reported in the table, we would like to point out that the follicles with a positive reaction for amyloid were made up of both chief cells and oxyphil cells. These follicles were mainly encountered in the periphery of the parathyroid gland.

Irrespective of the staining, two kinds of follicles could be differentiated: those with a uniform content and those with a stratified content, i.e. a laminated structure. A big, central, uniform nucleus was sometimes encountered in the latter [Fig. 1 (A) and (B)]. The detailed results of the various stains were as follows:—the follicles positive for amyloid reacted weakly to PAS whereas the negative ones reacted strongly. The follicles which reacted positively to Congo-red presenting a birefringence and yellow-green dichroism, when viewed in polarized light, contained a homogeneous substance although at times a laminated structure was visible.

The results obtained with Thioflavin T completely overlap the Congo-red results. In the case of Toluidine blue, carried at pH variable per unit from 1 to 4, the most pronounced metachromasia was obtained at pH 4. This metachromasia was obtainable only with follicles positive for amyloid; the colloid however was orthochromatic.

We were particularly interested in the DMAB method for tryptophan as this clearly differentiates between the two kinds of amyloid, i.e. Apud and immune (Pearse *et al.*, 1972). We would like to point out that the above mentioned staining properties were the same for the amyloid deposits of all parathyroid glands examined.

Out of the overall 191 follicles which were studied, 26 were positive (13.6%), 80 weakly positive or uncertain (41.8%), 85 negative (44.6%).

In the autofluorescence test it was not possible to find a definite concomitance between the autofluorescent follicles (772 out of 12 cases) and the follicles (belonging to the same sections) positive for amyloid (211 out of 12 cases).

The amyloid microdeposits encountered in other districts are shown in Table 3. These are indicated with a+ or a− irrespective of their proportions as we did not wish to assess these deposits quantitatively. Table 3 also shows the results obtained by decolorizing the slides positive for amyloid and restaining them with DMAB for tryptophan. Case no. 13 deserves special mention because the patient was a carrier of widespread cardiovascular amyloidosis and the degenerative process had also affected the parathyroid vessels (Fig. 2). It was therefore possible to compare in the same slide the behaviour of intrafollicular amyloid and vasal amyloid in relation to the various stains carried out.

We thus saw that the various stains presented overlapping results in the two kinds of deposits; the only stain which differentiated them was the method for tryptophan showing the vasal deposits positive and the follicular ones negative. The autofluorescence test was strongly positive in the vasal amyloid deposits (Fig. 3).

Discussion

The study of the substance in parathyroid follicles was mainly performed on glands obtained at operation from patients with hyperparathyroidism. In the case of normal glands, where the presence of these follicles is quite an old discovery (Herxheimer, 1926), the presence however of a substance with typical amyloid properties in some of these follicles is a recent discovery (Lieberman and De Lellis, 1973; Anderson and Ewen, 1974). However, studies carried out on normal glands by other authors have on the contrary produced negative results; thus Leedham and Pollock (1970), who did encounter this substance in 9 cases out of 88 with hyperparathyroidism, say that they did not encounter it in 20 normal parathyroid glands obtained at autopsy.

In his study performed on hyperplastic and adenomatous glands, Boquist (1973) affirms that he never obtained a positive reaction for follicular amyloid.

Hansson *et al.* (1974) define these deposits (which they studied in cases with primary hyperparathyroidism) as "corpora amylacea" and they include them in the same group as the lung and prostate deposits.

Table 4

	Leedham	Boquist	Hansson[a]	Anderson	Present study
Cases studied	88	106	108	106	13
Cases with amyloid (%)	9 (10.2%)	0	17 (15.7%)	32 (30.2%)	13 (100%)

[a] Mentions "Corpora Amylacea".

Data on quoted studies are presented in Table 4.

In addition to this we would like to point out that whenever a positive reaction for amyloid in parathyroid follicles is being looked for, with the specific aim to demonstrate it, it is encountered in 100% of the cases.

However we too noticed that in some sections there were no positive follicles for amyloid histochemical reactions, the reason being that the number of follicles varies enourmously from gland to gland.

Without studying all other sections we believe that it is not possible to deny the presence of positive follicles in every gland of an old person.

With this assumption we would now like to discuss briefly the histochemical characteristics of this amyloid.

The autofluorescence (after excitation in ultra violet light) of immunamyloid, absent in apudamyloid, and the positive reaction of immunamyloid to the DMAB method for tryptophan, negative for apudamyloid, are, according to Pearse (1972), the most reliable characteristics for differentiating between the two kinds of amyloid.

Moreover, according to Pearse, there are other reactions which show this distinction, even if less reliable, i.e. the demonstration for acid groups-COOH in side-chains (more positive in apudamyloid); the demonstration for radicals–S–S, and for Alcian blue at pH 2.5. However we did not seem it appropriate to carry out these reactions due to their weak discriminant power.

In the case of our material we must admit that it was not possible to assess exactly, "follicle by follicle", the presence or absence of autofluorescence of the amyloid because the follicles full of colloid appeared in their turn inconsistently autofluorescent. However after having counted the autofluorescent follicles in each case, it was noticed that the number of these follicles do not coincide, neither in value nor in tendency, with the corresponding number of follicles positive for amyloid demonstration carried out on the same preparations (see Table 5).

On the contrary it was possible to establish precisely the degree of positivity to the reaction for tryptophan in the follicles where the presence of amyloid had previously been determined.

We would like to point out that in the overall study of 191 follicles, 44.6% were negative, 41.8% weakly positive or uncertain and only 13.6% positive.

Therefore these data go to show that there is a small amount of tryptophan in the amyloid in parathyroid follicles. Consequently because autofluorescence is a property of tryptophan (Pearse *et al.*, 1972), we can say that it is frequently

Table 5

Case number	Congo red-dichroism follicle number	Autofluorescence (U.V. light) follicle number
1	—	7
2	10	9
3	—	1
4	44	55
5	7	30
6	5	75
7	10	104
8	1	—
9	35	42
10	—	9
11	73	420
12	26	21
13	—	5

absent in follicles with an amyloid content. This is proved by what was said for case no. 13 where both kinds of amyloid were present.

In fact, in this case, due to the dense vasal deposits of immunamyloid, it was possible to demonstrate—without any difficulty—the positive reaction for tryptophan as well as for autofluorescence.

As for intrafollicular amyloid here too it was only possible to demonstrate the negative reaction for tryptophan.

We are not able to say anything about the connection between the amyloid deposits which we studied and the APUD cellular systems because the presence of the latter in parathyroid glands has not yet been fully defined (Pearse *et al.*, 1972). Although other authors have entertained the possibility that this amyloid is produced by cellular elements of the APUD system, they have not yet come to a definite conclusion (Anderson and Ewen, 1974).

We saw that systemic deposits and microdeposits reacted differently to the test for tryptophan, depending on the type of organ. We thus noticed that all the microdeposits present in endocrine glands and contained in the follicles, or in the interstice of glands which do not tend to have follicles (islets of Langerhans, see Fig. 4), were unreactive for tryptophan. The contrary was noticed for the vasal deposits, even if in endocrine glands, as well as for the interstitial amyloid of the heart which turned out to be strongly positive.

Special mention must be made of the prostate and the encephalon. In the prostate there are various types of deposits i.e. vasal and utricular; the latter often constitute the so-called "corpora amylacea". As for the staining for tryptophan, the vasal and interstitial deposits resulted positive and also the "corpora amylacea", but to a lesser extent; negative or weak reaction was obtained for the amyloid present in prostatic secretion but not consisting of corpora amylacea.

As for the encephalon, we would just like to mention that the amyloid deposits in this organ vary extremely from a morphological point of view; however there was a strong positive reaction for tryptophan only in the vasal deposits.

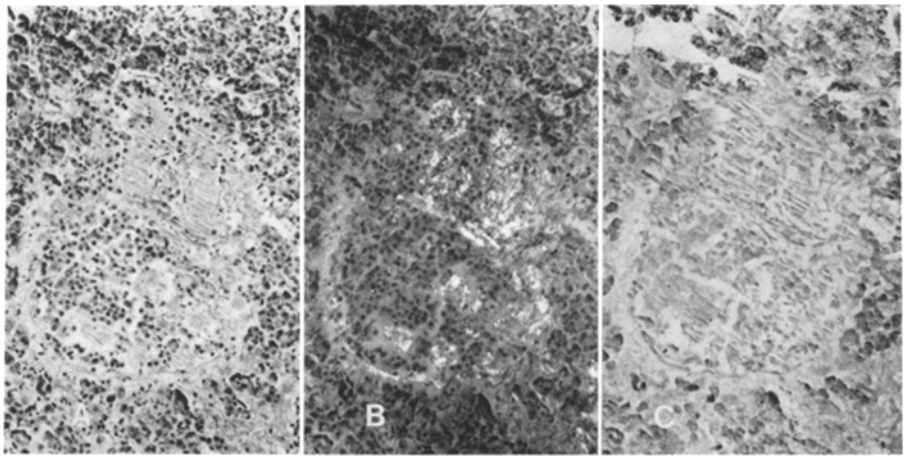

Fig. 4. (A) Aut. 1143/74 (Case no. 3); islet of Langerhans with amyloidosis stained with Congo red. (×40). (B) Same as previous viewed in polarized light. (C) Same islet decolorized and restained with DMAB method for tryptophan

On the basis of our study we can conclude by saying that according to the demonstration for tryptophan, two big categories of amyloid deposits are identifiable, i.e. the positive and unreactive ones for tryptophan. Often both of these deposits occur simultaneously in the same subject. The negative deposits, due especially to the fact that they are always encountered in glands, seem to be connected in their genesis to the so-called APUD system; viceversa the vasal deposits and partly the interstitial ones (e.g. in the heart) seem to be part of the so-called immunamyloid. As far as we are concerned, intrafollicular deposits in parathyroid glands is a typical example of amyloidosis of the APUD type.

Conclusion

1. The presence of follicles containing a substance with typical staining properties of amyloid in normal parathyroid glands of old persons is constantly discovered if looked for.

2. The amyloid in question possesses histochemical characteristics which identify it as apudamyloid. Possible vasal and interstitial amyloid deposits in persons with generalized amyloidosis present characteristics typical of the so-called immunamyloid.

3. Systemic deposits and microdeposits present in old persons may simultaneously belong to both systems. In particular deposits which affect the endocrine glands have histochemical characteristics typical of apudamyloid; vasal and interstitial deposits have characteristics typical of immunamyloid. Both types of deposits can occur simultaneously in the same persons.

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