Wagner (1), in 1866, first reported colloid milium in a 54 year old woman who showed lesions on the forehead, cheeks and nose. In patients with colloid milium, the involved skin is usually hyperpigmented, thickened, furrowed, and covered with multiple 0.5—5 mm dome-shaped, discrete papules. The shiny, pink or orange to yellowish white translucent lesions have been likened to vesicles, but are firm and only after considerable pressure can a clear to yellow mucoid substance be expressed from the papules. The lesions involve sun exposed sites including the dorsum of the hands, web between the thumb and index finger, knuckles, forearms, malar regions, nose, ears, sides and back of the neck, and temporal areas. In 100 years extending from 1866—1966, approximately 100 cases of colloid milium have been reported in the literature, but as yet there is no uniform agreement with regard to the nature of colloid.

The purpose of this paper is to record our histopathologic, histochemical, fluorescent microscopic, and polariscopic observations regarding the morphologic features and composition of colloid.

MATERIAL AND METHODS

This report is based on observations of one or more biopsies from 5 patients with typical lesions of colloid milium. The specimens were obtained with a 4 or 6 mm cutaneous punch and fixed in 10% neutral buffered formalin. With the exception of 1 specimen (courtesy of P. J. Alfaro, M.D., Bayamon, Puerto Rico), all tissues were processed for paraffin-blocked sections. Multiple sections stained with hematoxylin and eosin (H & E) were examined in all cases, and additional biopsy sections from 3 of the patients were prepared by the following methods: periodic acid-Schiff (PAS) reaction, with and without diastase digestion; colloidial iron reaction, with and without bovine testicular hyaluronidase digestion for 1 hour at 37 C; Movat's pentachrome I stain (2); alcian blue pH 2.5 and 0.4 (3, 4); aldehyde-fuchsin pH 1.7 and 0.4 (4), with and without elastase digestion (5); Snook's reticulum stain; phosphotungstic acid hematoxylin stain (PTAH); Prussian blue reaction for iron; Fontana-Masson stain for argentaffin granules; thioflavine T fluorescent stain (6, 7); Congo red; alkaline Congo red method (8); crystal violet amyloid stain; methyl violet stain for amyloid (9, 5); toluidine blue (4); and Giemsa stain. The crystal violet and methyl violet stained sections were mounted in Highman's Apathy gum syrup (5) which tends to prevent bleeding and gives a more permanent preparation. Formalin-fixed tissue from 1 patient with colloid milium was used for preparing frozen sections of unstained sections, utilizing the above methods, and for oil red O and Sudan black B fat stains. The majority of the wet and paraffin-blocked tissues were sectioned at 5 μ, but some were cut 10—40 μ thick and stained by the listed methods. All mounted tissue sections were examined polariscopically (5, 10) utilizing polarizer and analyzer lenses. The thioflavine T and Congo red stained sections were examined microscopically for fluorescence with a Leitz Ortholux microscope equipped with an Osram HBO 200 W light source, a UGI excitor, and an ultraviolet absorbing barrier filter. The Congo red stained sections were also examined with the same fluorescent microscope system, but utilizing a darkfield condenser (11). With the exceptions given, the procedures were carried out as outlined in the “Manual of Histologic and Special Staining Techniques” (12).

For comparative purposes, skin sections prepared by the various techniques were studied from 4 adults with lichen amyloidosis and 1 adult with primary systemic amyloidosis cutis.

CLINICAL DATA

The biopsy specimens came from 5 Caucasian men and all were clinically diagnosed as having colloid milium. Their ages were 32, 33, 36, 49 and 65 years respectively. The 2 patients who were 32 and 36 years old came from Philadelphia, Pennsylvania; and the remaining 3 had spent most of their lives in the region of Columbus, Georgia; Bayamon, Puerto Rico; and Rio de Janeiro, Brazil. For occupational reasons, 4 of the 5 patients had a greater than average amount of exposure to sunlight. All patients showed multiple lesions.
typical of colloid milium and these were limited to the dorsum of the hands in 2; the malar regions in 1; 1 had involvement of the malar regions and dorsum of the hands; and the remaining patient had discrete papules on the dorsum of the hands and posterior neck. Biopsies from 4 of the patients came from the hands, and the patient with lesions limited to the face had a biopsy taken from the left zygomatic region. The stated duration of the asymptomatic lesions prior to consulting a physician varied from 1 to 6 years. All patients were in good general health, but 4 of the 5 showed actinic skin changes.

**Histopathologic Observations**

Microscopically, H & E stained biopsy sections from the 5 patients showed similar features. The epidermis was elevated at irregular intervals with hyperkeratosis, intact granular layer, atrophy, hyperpigmentation of the basal layer, and effacement of the rete ridges overlying hyaline masses in the papillary corium (Fig. 1—top). Some sections showed peripheral collarette formation which appeared to localize the colloid to broadened dermal papillae (Fig. 1—bottom). Generally, a grenz zone of relatively uninvolved connective tissue separated the epidermis from the colloid de-
posits, but focally, the hyaline material extended to the dermoeipidermal junction producing changes of liquefaction degeneration. The colloid appeared as an acidophilic, amorphous, smooth, uniform substance replacing the connective tissue (Fig. 1). The homogeneous masses were frequently fissured with cleft-like retraction spaces (Fig. 1). Spindle and stellate shaped fibroblasts are scattered throughout the colloid and these cells tend to arrange their long axis along the lines of fissuring (Fig. 2). Papillary capillaries course through and adjacent to the hyaline masses, and their walls sometime show infiltration by the acidophilic substance (Fig. 3—bottom). Vascular ectasia is usually seen in the grenz zone and beneath the colloid, and some of the dilated blood vessels extend into the clefts between the hyaline material (Fig. 3). Occasionally, a few lymphocytes, histiocytes, and rarely plasma cells were present laterally and at the base adjacent to the collections of colloid. Sections from 4 out of the 5 patients showed basophilic changes of solar elastosis at the periphery and beneath the colloid substance (Figs. 1—bottom and 5). Some sections showed the abnormal elastotic changes completely surrounding and separating aggregates of colloid, but the morphologic appearance and staining characteristics of the 2 substances were distinctly different (Fig. 6—left). In a few areas, small clumps of basophilic elastotic tissue appeared entrapped in the hyaline substance, but this may be misleading from the plane of single sections because deeper in the block continuity was usually established with larger masses of solar elastosis at the base or periphery of the colloid (Fig. 6—left).

Histochemical Observations

The significant results of histochemical studies were as follows: The Fontana-Masson stain for melanin showed an abundance of pigment in the basal layer of the epidermis, and lesser amounts free and within melanophages in the region of the colloid deposits. No hemosiderin was demonstrated with the Prussian blue reaction for iron. The colloid was PAS-positive and diastase resistant (Fig. 3), showed affinity for Congo red, stained metachromatically a purplish to red color with methyl violet and crystal violet (Fig. 4), appeared pink with toluidine blue, and exhibited a variable amount of colloidal iron and alcian

Fig. 2. High power magnification showing spindle and stellate-shaped fibroblasts throughout the colloid. Giemsa, × 655.
blue (pH 2.5) positive material separating, coating, and surrounding the hyaline deposits. This latter substance is bovine testicular hyaluronidase labile and negative with alcian blue (pH 0.4), identifying it as hyaluronic acid. Some of the colloid masses showed uniform staining with aldehyde-fuchsin pH 1.7 and 0.4, probably indicating the presence of sulfated acid mucopolysaccharides. Elastic tissue in areas of solar elastosis, and a moderate increase in mast cells were also best demonstrated with aldehyde-fuchsin (Fig. 5). Elastase digestion removed the aldehyde-fuchsin reactive elastic tissue and abnormal elastic fibers of solar elastosis, but had no effect on the colloid or mast cells. Some of the colloid stained similar to fibrin with PTAH, and this suggests the presence of fibrin or fibrin precursors as a component of colloid. The PAS-positive and diastase resistant material may in part be due to the presence of fibrin, serum proteins, and complex mucosaccharides bound to proteins through covalent linkages. The pentachrome stain colored the colloid a pale grey to light yellow which was tinctorially distinct from collagen and elastic tissue. Occasionally, narrow septae occupied by blood vessels, collagen fibrils, elastic fibers, and solar elastotic tissue completely surrounded and divided small accumulations of colloid. The areas of solar elastosis surrounding some of the colloid masses was striking (colored black

Fig. 3. Top. The colloid is PAS positive. PAS, × 55. Bottom. The PAS-positive and diastase resistant colloid shows a striking contrast with the epidermis, grenz zone, and underlying corium. At the left, the colloid shows infiltration of the wall of a small blood vessel. PAS with Diastase, × 66.
to red with resorcin-fuchsin and purple to blue-black with aldehyde-fuchsin) and emphasize the general absence of elastic fibers and collagen in the colloid substance (Fig. 5). Snook's silver stain demonstrated a granularity of the colloid and a delicate network of argyrophilic fibers surrounding individual collections of colloid (Fig. 6—right). This feature may account in part for the characteristic fissuring seen in routine H & E sections (Fig. 1). It is likely that the cleft-like spaces are artefacts from tissue processing, and occur along normal lines of cleavage which in situ contain blood vessels, fibroblasts, reticular fibers, and ground substance material. Sections prepared with the Giemsa stain were particularly good for demonstrating the spindle-shaped or fusiform fibroblasts (Fig. 2). The oil red O and Sudan black B stains for lipids showed a moderately positive staining reaction which uniformly colored the colloid. With the exception of the fat stains, the results observed in paraffin sections were usually preferable for most of the histochemical studies performed.

Sometimes, there was enhancement of staining with Congo red in 10–15 μ paraffin sections, and when longer periods of staining were used (sometimes overnight or even 24–72 hours).

**FLUORESCENT MICROSCOPY OBSERVATIONS**

Frozen and paraffin sections stained with thioflavine T showed a uniform yellow to green fluorescence under ultraviolet light and contrasted with the dull gray of the adjacent epidermis and connective tissue. Paraffin sections cut at 15 μ gave optimal results, and this is apparently a quantitative factor of enhanced fluorescence in the presence of a greater volume of organized structure. Congo red stained sections of colloid appeared pink under brightfield fluorescence microscopy. When a darkfield condenser was used, colloid stained with Congo red showed a moderate red fluorescence and stood out well when contrasted with the gray connective tissue fibers. Paraffin sections 10–15 μ, stained with Congo red gave the most satisfactory results.
Polariscopic Observations

Congo red stained sections of colloid milium examined under polarized light show areas of birefringence, dichroism and even some degree of trichroism. Dichroism refers to a change in color that varies with the plane of polarization. The birefringent color of colloid can be changed from yellow with shades of orange to bluish-green by rotating the polarizer or analyzer lenses while keeping the deposits under direct observation. Paraffin or frozen sections 10-15 μ enhanced the prominence of birefringence of the colloid deposits and could even be demonstrated in unstained frozen sections of those showing only minimal affinity for Congo red. Paraffin sections cut at 15 μ and stained with thioflavine T showed prominent bright silvery birefringence of the colloid, but dichroism was minimal. Thick sections stained by techniques other than Congo red and thioflavine T also exhibit areas of birefringence, but dichroism is essentially absent other than the color of the dye usually seen in the tissue sections. Toluidine blue stained paraffin sections 15 μ thick showed a striking silvery birefringence with a bluish sheen of the colloid deposits. The phenomenon of dichroism and trichroism is best illustrated with Congo red staining of 30 μ sections, and the prominence of color changes seem directly related to the affinity of the dye for the colloid deposits. Thick sections of colloid probably enhance birefringence quantitatively. Morphologically, the birefringent components of colloid appear granular and fibrillar, and probably represent an abnormal scleroprotein different from collagen and elastin.

Comparative Observations with Amyloidosis Cutis

The significant results of histopathologic, histochemical, fluorescent and polarization studies were as follows: Cutaneous amyloid and colloid deposits show similar characteristics with some minor differences. A common feature is that amyloid, like colloid shows variability of staining reactions, particularly with Congo red and toluidine blue. Methyl violet and crystal violet stained sections of amyloidosis cutis show the amyloid deposits have a tendency to appear pink to red, whereas colloid is usually purple with elements of red. The PAS-stained sections of amyloidosis cutis usually show a less intense color reaction than seen for colloid. The PTAH stain is essentially negative for amyloid, but demonstrates some reactive material in the colloid deposits. Oil red O stained frozen sections of lichen amyloidosis were essentially negative and the Sudan black B reaction was only weakly positive, as compared to a moderately positive staining of colloid by these methods.

Thioflavine T stained sections of amyloidosis cutis tend to fluoresce a yellowish-green similar to colloid, but this was a variable feature. In general, the thioflavine T stained sections of primary systemic amyloidosis cutis showed the most prominent fluorescence, but some 15 μ paraffin sections of colloid exhibited a brilliant yellow to green color. The thicker sections of 10-15 μ were usually best for demonstrating fluorescence in the thioflavine T stained sections of lichen amyloidosis.

Birefringence and dichroism of amyloid deposits were minimal to absent in Congo red stained 5 μ paraffin sections of lichen amyloidosis, whereas, the phenomenon was striking in primary systemic amyloidosis cutis. Sections 10-15 μ stained with Congo red and thioflavine T enhanced birefringence in lichen amyloidosis and this was a similar feature described for colloid milium. The characteristic of trichroism under polarized light is a difference seen in colloid milium as compared to the yellow to green dichroism of amyloidosis cutis.

Discussion

Current opinions in the literature are varied regarding the exact composition of the hyaline material demonstrated in routine H & E stained sections of colloid milium. Some interpret colloid as abnormal elastic tissue (13, 14), others consider it a form of collagen degeneration (15-17), while there are those that feel it is an alteration involving both collagen and elastin (18-21). Percival and Duthie (22) favored that colloid was not a connective tissue change, but a protein infiltration similar to amyloid. Subsequently, Zoon, Jansen and Hovenkamp (23), and Becker and Wilson (24), by paper chromatography showed that colloid has an amino acid composition identical with serum proteins. They (23, 24) dem-
onstrated that hydroxyproline was absent in colloid and serum, but present in collagen and elastin, and concluded that colloid originates from serum proteins. Woolridge and Frerichs (25), in 1960, reported a 12 year old boy with a clinical type of amyloidosis involving the cheeks, nose, and sides of the neck. They (25) interpreted the deposited material as protein in character, and that the patient showed features of amyloidosis cutis and colloid milium. Woolridge and Frerichs (25) speculated that future investigations may prove colloid and amyloid are closely related substances, but finally classified their patient as cutaneous amyloidosis. Sullivan and Ellis (21) reported an unusual case resembling that of Woolridge and Frerichs (25), and mentioned that there are many clinical and histologic similarities of colloid and amyloid.

Polariscopic studies have demonstrated that amyloid stained by Congo red exhibits birefringence and dichroism (7, 10, 26—31). These characteristics indicated an underlying organized structure might be present, and suggested that a study of the ultrastructure might be fruitful. In 1959, Cohen and Calkins (32), demonstrated that amyloid had a finely fibrous component. Hashimoto, Gross and Lever (33), showed the amyloid in lichen amyloidosis consisted of filaments embedded in an amorphous ground substance, and concluded that the material is an abnormal secretion product laid down by fibroblasts. Gafni, Merker, Shibole, Sohar and Heller (34), studied a scalp amyloid tumor and demonstrated fibroblasts showing signs of active secretion in a sea of amyloid filaments. They (34) thought their observations supported the concept that amyloid is a variant of one of the normal scleroproteins. In a recent review article, Cohen (31) indicated that fine amyloid fibrils are a consistent finding in all types of amyloidosis, and this substance is formed locally by reticulo-endothelial cells in human and experimental animal tissues. The amyloid fibrils are protein in nature, probably containing small amounts of carbohydrate, and are embedded in ground substance, which itself may be normal or abnormal (31).

Piredda (16) examined 10—12 μ sections of colloid milium polariscopically and concluded that birefringence was essentially absent. Review of the article (16) seems to suggest there were a few scattered fibrillar elements showing a soft residue of birefringence which faded out toward the center of the colloid nodule. Electron microscopic studies by Piredda (16) showed the amyloid in lichen amyloidosis substance mixed with fibrillar elements which appeared as intensively altered collagen. Piredda (16) concluded that colloid represented a progressive degeneration of collagen and is not a foreign substance deposited in the skin with secondary morphologic changes.

Our observations of birefringence and color changes (dichroism and trichroism) in sections of colloid milium indicated to us several years ago that colloid was probably structurally similar to amyloid, and that additional ultrastructure studies would demonstrate this to be true. Personal communication from Hashimoto and Bereston (35) regarding preliminary electron microscopic studies of colloid milium are as follows: colloid appears to be amorphous with amyloid-like filaments. A striking feature is that every small or large island of colloid is surrounded by fibroblasts which show intracytoplasmic precursor material. Higher resolution studies will be necessary, but the dimensions of the filaments with some beaded pattern in colloid milium are compatible with similar features demonstrated for amyloid (33).

Histochemically, with only minor differences, colloid is similar to amyloidosis cutis. Our study and those of many others on amyloidosis cutis document the variability in the staining reactions for amyloid. Variation in results of staining colloid and amyloid can occur from laboratory to laboratory, and even from numerous technical problems in the same laboratory. Technical problems undoubtedly are responsible for some conflicting results reported for colloid and amyloid, but a consideration is that these substances are of variable composition which will alter the staining reactions from case to case, and in different tissues in the same patient. Congo red has been the most variable stain for colloid and amyloid in our laboratory, but longer staining and/or thicker tissue sections have usually given positive results. Even with minimal staining reaction, Congo red enhances birefringence and is mainly responsible for color changes exhibited by colloid and amyloid under polarized light. The phenomenon of
dichroism occurs as a result of altering the plane of light by rotating the polarizer lenses while examining Congo red stained sections of colloid or amyloid. Puchetler, Sweat and Levine (7) concluded that Congo red staining of amyloid is by a nonionic binding by hydrogen bonding between the hydroxyl groups of amyloid and the amino groups of the dye. Gueft and Ghidoni (36) suggested that Congo red staining of amyloid may be analogous to cotton fiber staining, and the double fibril configuration may physically sequester the flat Congo red molecule, forming an inclusion compound. It is probable that Congo red and other dyes show affinity for colloid similar to amyloid because of the fibrillar nature of these 2 substances. Birefringence of thioflavine T stained sections of colloid and primary systemic amyloidosis cutis was striking. One can speculate that thioflavine T may show affinity for the fibrils of colloid and amyloid similar to that suggested for Congo red. There is little doubt that the organized structures demonstrated by fluorescent microscopy of thioflavine T stained sections of colloid and amyloid account for the brilliant birefringence seen when the same sections are examined under polarized light. Toluidine blue, crystal violet and methyl violet stain colloid and amyloid metachromatically principally because of the presence of hyaluronic acid, and probably to a lesser degree because of sulfated acid mucopolysaccharides. The reason for toluidine blue enhancing birefringence of colloid under polarized light even after fading of the metachromasia is difficult to explain, but one of the reasons for the affinity that all the dyes show for colloid may be physical rather than chemical because of fibrillar structures.

Staining of colloid with thioflavine T has generally been reported as giving negative results (6, 21). Heyl (37), in 1966, reported 4 cases of clinically and histologically typical colloid milium which stained positive with thioflavine T. Heyl (37) mentioned the negative results reported by Kurban (6) and thought the contrasting results could be explained by use of different optical systems or variation in the pre-staining treatment of tissue blocks. Our results with thioflavine T showed a yellow to green fluorescence of colloid deposits and we agree with Heyl (37) that fluorescence is the criterion for judging the material positive, and not the color or color intensity.

Regarding the origin of colloid, it is our opinion that this substance is closely related to amyloid occurring in the skin, but the etiology of both diseases remains a mystery. Whatever the causative factor or factors are in colloid milium, they undoubtedly stimulate the dermal fibroblasts to function in an abnormal way leading to the synthesis of a pathologic scleroprotein, analogous to its normal counterparts, collagen, elastin and keratin. Studies (38—40) indicate that dermal fibroblasts are the source of acid mucopolysaccharides in the ground substance and precursors of collagen and elastic tissue. In pathologic conditions such as colloid milium and amyloidosis cutis, fibroblasts are influenced to produce a pathologic scleroprotein and greater amounts of acid mucopolysaccharide (particularly hyaluronic acid) at the expense of the normal formed elements, and this results in partial to complete replacement of reticulum, collagen and elastin. Like amyloid, colloid can probably be classified as a rare in vivo solid pathologic protein which causes human disease. The presence of an amino acid spectrum in colloid milium which is similar to serum proteins is easily explained on the basis of periodic deposition of blood elements in areas of hyaline formation. Hyaline infiltration of small blood vessels in the region of colloid deposits undoubtedly weakens these structures resulting in fragility and escape of serum proteins. This feature is analogous to that occurring in lesions of primary systemic amyloidosis cutis in which the blood vessel walls are infiltrated by amyloid resulting in vascular fragility, with gross evidence of petechiae and ecchymoses in the region of the papules and nodules.

The influence of sunlight as the cause of colloid milium is frequently mentioned in the literature and textbooks of dermatology. The absence of solar elastosis in one of our patients, others reported in the literature, and the rare occurrence of cutaneous colloid degeneration on covered parts of the body (15) tends to minimize sunlight as a primary cause of colloid milium. Because of the striking distribution of colloid milium to sun-exposed areas of the body and its more common occurrence in Southern regions, it is possible
that actinic rays may act as a promoting factor after unknown initiating agents have triggered the disease.

SUMMARY

Our histopathologic, histochemical, fluorescent microscopic, and polaroscopic studies strongly suggest that colloid is similar to amyloid. Our observations of fluorescent staining of colloid with thioflavine T and birefringence of the hyaline substance under polarized light indicate an organized fibrillar structure. It is our opinion that colloid represents an abnormal seleroprotein synthesized by dermal fibroblasts, and this is a solid pathologic process. Our observations of fluorescent staining tor after unknown initiating agents have suggested that actinic rays may act as a promoting factor. Our observations of fluorescent staining of colloid with thioflavine T and birefringence of the hyaline substance under polarized light indicate an organized fibrillar structure. It is our opinion that colloid represents an abnormal seleroprotein synthesized by dermal fibroblasts, and this is a solid pathologic process.

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