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The Lipopigments in Human Brain Tissue Necroses

II. Hemoceroid*

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Summary. A serial and comparative histochemical examination of lipopigments in human brain tissue necroses led to a more exact characterization of a chromolipid called "hemoceroid". It arises only in necroses with hemorrhages and not in anemic necroses nor in subdural hematomas. Its histochemical properties differ from those of all developmental stages of ceroid. An ageing phenomenon is absent. The very intense autofluorescence of the thick, clotted pigment ist striking. Hemoceroid seems to arise extracellularly and is absorbed secondarily by macrophages.

Key words: Special chromolipids – Histochemistry – Fluorescent microscopy – Hemorrhagic brain tissue necroses

Besides blood pigments and ceroid, special lipopigments, differing from presently known pigments, are formed selectively in hemorrhagic brain tissue necroses. These chromolipids are characterized particularly by their large size, special color in sections stained by luxol fast blue and intense autofluorescence. We labeled them "hemoceroid" (Schröder 1978; Schröder and Reinartz 1979).

In the present study this type of pigment is examined with regard to the occurrence and possible time-dependent alteration of histochemical characteristics, and it is compared with ceroid (Schröder 1980).

Material and Methods

The material examined and the histochemical methods used are the same as those reported in the first section of the study (Schröder 1980).

In addition, a series of 90 necrotic fields from this material was examined by fluorescent microscopy, whereby the same sections stained by HE and luxol fast blue and some unstained sections for comparison, were used to identify the hemoceroid. The cases had a lesion aged 1 day to 8 months. Also, 13 subdural hematomas with intervals of 12 days to 18 months were examined by fluorescent microscopy. We used a Leitz "Ortholux 1" microscope (Leitz, Wetzlar, FRG) with a mercury lamp and a BG-12 blue, respectively UG-1 ultraviolet, exciter filter in combination with corresponding absorption filters.

Results

Appearance and Location

Hemoceroid was conspicuous by an inherent yellow straw color, by its homogenous wax-like quality, and in luxol preparation by a typical light green color, which other pigments did not show. The substance often consisting of subunits appeared as clusters with diameters ranging from 3–20 μm. The irregular masses filled nearly the total cytoplasma of macrophages. Frequently, these cells were especially large and multinucleated. Already due to their size alone, this pigment stood out among the fine ceroid granules. Furthermore, hemoceroid exhibited very intense autofluorescence, which definitely exceeded that of ceroid (Fig. 3 a, b). This permitted fluorescent microscopy as a second method suitable to detect hemoceroid.

In intracerebral hemorrhages, however, the pigment was not limited to the cells of the phagocytic zone, 200–400 μm in thickness. Hemoceroid also appeared at times in relatively abundant amounts in the inner part of massive hemorrhages at depth of more than 1 mm, where macrophages could be found in only small numbers. Here a part of the hemoceroid seemed to lay free in the detritus without recognizable connection to macrophages. This extracellular location was not only observed in early states, but also later on.

In necroses with only small hemorrhages the pigment could also be found in close relation to the hemorrhages.

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Occurrence

Hemoceroid was observed in hemorrhagic necroses or in larger hemorrhages and was absent in anemic necroses and subdural hematomas.

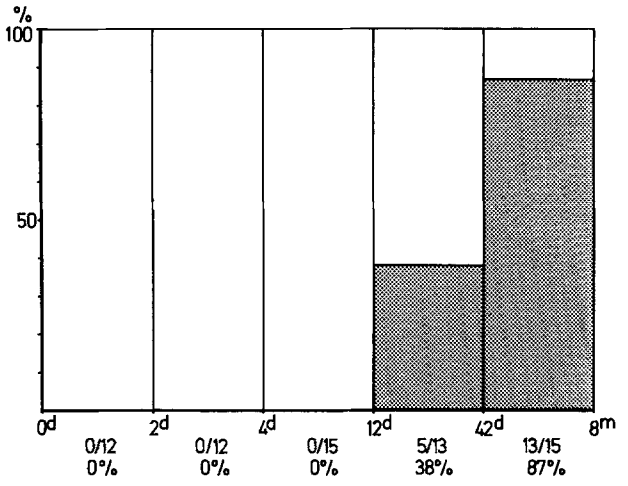


Fig. 1. Proportion of cases with hemoceroid (shaded columns) to all fluorescence-microscopically examined cases in relation to different times. In the lower rows the absolute numbers in these groups are given in the form of proper fractions as well as the percentages. d = days, m = months

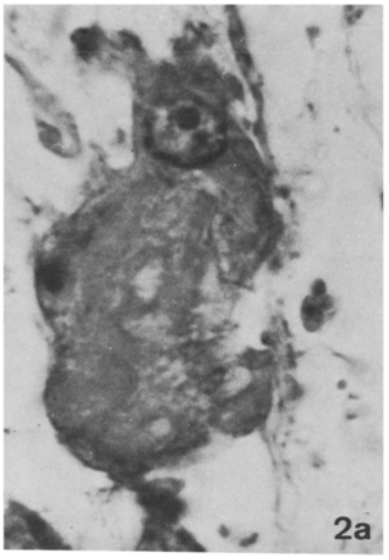
Hemoceroid could be identified first after 12 days and was evident for 8 months; i.e., it disappeared at practically the same time as the rest of the detritus (11 months). The frequency of the pigment tended, however, to vary. Sometimes one or two specimens were found in the lesion; in other cases, over 100 were estimated with no correlation to the size of the necrosis. Generally, however, the number of the coarse pigment clusters was substantially greater in larger hemorrhages than in necroses with smaller hemorrhages. However, in spite of systematic examination of the preparations, the pigment could be identified within the mentioned time in only a portion of the cases. By fluorescent microscopy it was found within that time in 10 of 14 cases with larger hemorrhages (71%), and in eight of 14 with hemorrhagic necroses (57%); however, when the luxol preparation was examined by white light in only nine of 45 cases with both types of necroses. This difference is probably due to the fact that by the last method, only larger objects can be adequately identified.

As shown in Fig. 1, the frequency of cases with hemoceroid increased within the period of 12 days to 8 months. The sufficient number of cases examined with shorter intervals suggests that the pigment can hardly appear much earlier than 12 days.

Table 1. Synopsis of occurrence and histochemical and physical properties of hemoceroid compared with the developmental row of ceroid reported in the first part of the study (Schröder 1980)

	Ceroid				Hemoceroid
	A	B	C	D	
Occurrence in					
gray matter	44^h-4^d	51^h-8^d	–	8^d-4^{yr}	12^d-8^{mo}
white matter	44^h-55^d	51^h-27^d	15^d-4^{yr}	52^d-4^{yr}	
Inherent color	yellow	yellow	brownish-yellow	yellow-brown	straw-yellow
Luxol fast blue	+++→+ green	0/(+) yellow-ocher	+/++ khaki	0 brown	(+)/+ light-green
Sudan black B	+	+/++	++	++/+++	+++
Ziehl-Neelsen	(+)/+	(+)/+	+	++	++
PAS	+++	+++	+++	+++	(+)
PfAS	+++	+++	+++	+++	++
Chrome hematoxylin	+++	+++	+++	+++	(+)
Toluidine blue, pH 4.2	0	+	+/++	++/+++	(+)
Argentaffine reaction	0	0	+	+	0
Autofluorescence	+/++	+/++	+/++	++	+++
Fluorescence, HE stain	(+)	(+)	+	+	+++
Neutral red staining	+++	+++	+++	+++	+++
Neutral red-fluorescence	0	0	0	0	++
Birefringence	0	0	0	0	0
Amido black 10 B	0..+	+	0..++	0..++	0
DDD	0	0	0	0	0
Hale	0	0..+	0..++	0..+	0
Prussian blue	0	0	0	0	0

h = hours, d = days, mo = months, yr = years; 0 = no reaction



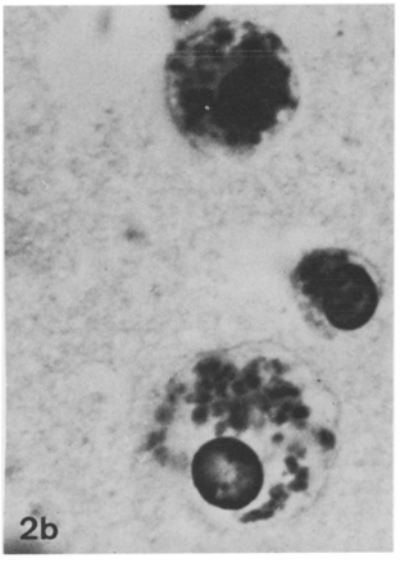
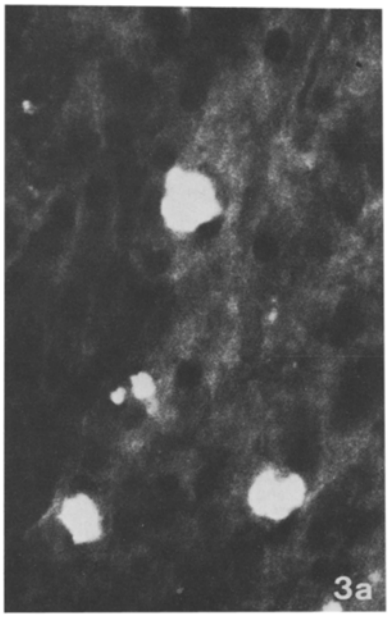


Fig. 2. a A large macrophage nearly filled with hemoceroid stained only weakly. Intracerebral hemorrhage 2 months before death. **b** Typical intense staining of ceroid-granules in macrophages of an anemic infarct 2 months old. Chrome hematoxylin, × 1,270



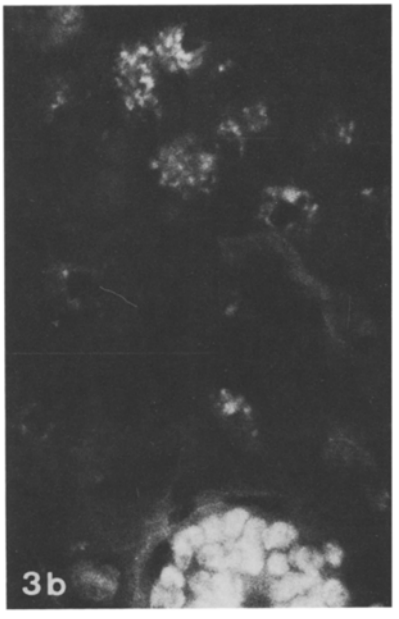
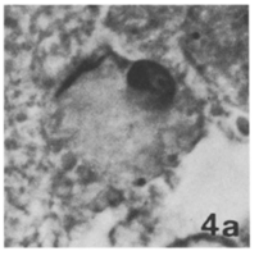


Fig. 3. a Very intense fluorescence of hemoceroid in a hemorrhage 2 months old. **b** Distinct, but weak fluorescence of ceroid granules in comparison with the intravasal erythrocytes (lower right corner) in a cystic necrosis 2 months old. HE, × 530. **a** and **b** are both photographed under the same exposure conditions



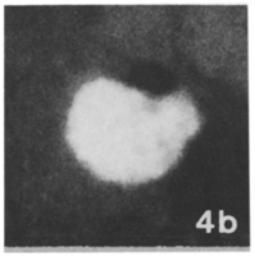


Fig. 4a,b. The same macrophage — **a** by white light, **b** by UV — filled with hemoceroid. HE, × 1,270

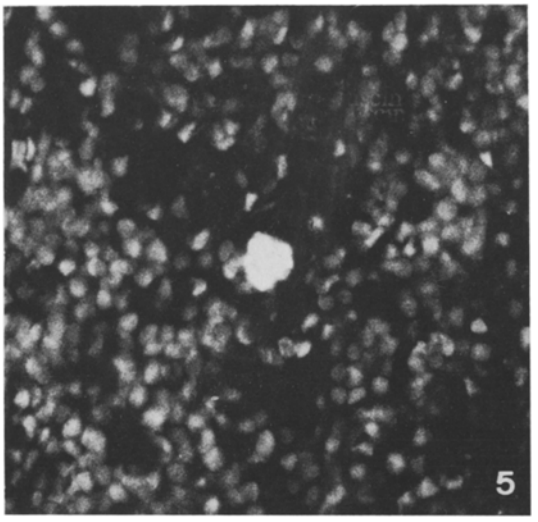


Fig. 5. Bright fluorescence of hemoceroid in relation to fresh erythrocytes. HE × 530

Histochemical and Physical Qualitites

In contrast to ceroid pigment, hemoceroid remained characteristically unchanged during the entire period of its existence of 12 days to 8 months. The results are presented in Table 1. They are contrasted with the findings of the four developmental stages of typical ceroid (Schröder 1980). The varying thickness of the different-sized pigments was taken into account when assessing the reaction intensity.

The methods employed permitted clear differentiation of the hemoceroid from other phagocytic pigments. Certainly, with Sudan black, Ziehl-Neelsen, and neutral red staining, similarity of color intensity existed with pigment D. In these preparations size, homogeneity, and fluorescence of hemoceroid as well as characteristical positional relationships in control sections had to be utilized to assist in identification. However, other reactions (PAS, PfAS, basophilia, chrome hematoxylin, argentaffine reaction) turned out in part so weak or negative – especially in comparison with the ceroid pigments C and D which appeared simultaneously – that a definite differentiation was possible (Fig. 2 a, b). The very intense primary fluorescence should be emphasized (Fig. 4 a, b), appearing of white color with a weak yellow component in blue light and of white color with a weak green component in ultraviolet exciting lightening. The difference from typical ceroid pigments was especially apparent when sections stained by HE were examined by fluorescent microscopy. Here, while the brightness of the fine-granular lipopigments hardly attained that of the erythrocytes (Fig. 3 b) the clumps of hemoceroid fluoresced much stronger than the erythrocytes (Fig. 5). Transitional forms between the two kinds of pigments were not identified.

Discussion

Exclusively in hemorrhagic brain tissue necroses we could single out a pigment, which we have labeled "hemoceroid" (Schröder 1978; Schröder and Reinartz 1979) according to Hartroft (1953). Hartroft described a pigment in hemorrhagic atheromatous vessel lesions that differed from ceroid in non-hemorrhagic plaques. It was characterized by intensive acid fastness, sudanophilia, and strong autofluorescence. Unfortunately, further differentiation has not been made. Whether or not it is identical to cerebral hemoceroid, which also exhibits these properties, is not certain. Because we have found the entire reaction pattern of the cerebral hemoceroid in pigments of hemorrhagic fatty tissue necrosis as well (Schröder and Reinartz 1979), it is probably identical to the atheroma pigment and its occurrence is certainly not confined to the brain.

When compared with typical ceroid forms A to D (Schröder 1980), hemoceroid exhibits a number of differences besides the close connection with bleeding. It forms relatively late, after 12 days, becomes gradually more frequent, and disappears at 8 months. During this time the pigment exhibits constant staining and fluorescence pecularities. While typical ceroid reaches the final stage in 8 days in macrophages of the cortex and in 2 months in macrophages of the white matter, hemoceroid seems to lack a comparative ageing process. It differs further in its thick, clotted quality.

Histochemically, agreement between hemoceroid and the simultaneously occurring ceroids C and especially D can be determined by the deep Sudan black and Ziehl-Neelsen staining. The intensive neutral red staining also speaks in favor of a lipopigment (Haitinger 1959). Moreover, proof is lacking here as to a participation of proteins (Amido black, DDD) and acid mucopolysaccharides (Hale's reaction) in the pigment substance. In contrast, the inherent color of hemoceroid is much lighter and the remaining reactions turn out weaker so that speculations about a different chemical composition are possible.

With the performic acid-Schiff reaction the ethylene-type double bonds of unsaturated fatty acid molecules can be identified (Lillie 1952); with the PAS method their oxidation products can be, too, in the form of adjacent hydroxyl-groups (Gedigk and Fischer 1959). These groups existing in larger amounts in the ceroid A to D apparently are diminished distinctly in hemoceroid. The absence of basophilia shows further that the higher oxidation levels of the unsaturated fatty acids (i.e., keto-acids), the increase of which can be easily followed in the pigments A to D, are for the most part missing. The lacking reduction of ammoniacal silver-nitrate solutions (Masson-Hamperl) likewise indicates a lack of active groups, especially carbonyl and ethylene groups as well as epoxides (Gedigk and Pioch 1965). To be interpreted in the same sense is the result of the chrome hematoxylin staining, of which until now only little use has been made in pigment research (Takada 1970; Takahashi et al. 1976, 1977 a, b). Hereby, because of the preceding oxidation with potassium permanganate, acid groups arise which react with the alkaline dye (Müller 1954). The findings, therefore, unanimously indicate a comparatively low proportion of oxidizable groups in hemoceroid. This explains why the pigment does not change with age.

The particularly intense primary and secondary fluorescence after neutral red staining is considerably more difficult to interpret, as little is known still about its causes (De Lerma 1958; Wolman 1975). The presence of especially numerous conjugated double bonds in hemoceroid can probably be denied. Also, the involvement of aromatic compounds can hardly be assumed in view of the missing protein part. For the same reason, the assumption that a binding of protein-

amino groups with carbonyl groups of fatty acids as the cause of fluorescence does not hold (Fletscher and Tappel 1971). Because of the close connection with hemorrhage, the participation of porphyrin, the fluorescent property of which is known, can be discussed. However, porphyrins produce a red or orange fluorescence color in UV light, hemoceroid on the contrary exhibits a white color. More worthy of consideration seems dependence on the degree of polymerization.

Hemoceroid does not appear in subdural hematomas. Therefore, deteriorated blood cannot represent the sole source. Another factor originating in the surrounding tissue must be present for the formation of the pigment. Worthy of consideration in this context are the free, highly unsaturated fatty acids, which have been identified in brain necroses (Lindlar and Güttler 1966; Lindlar and Lorenz 1968). They diffuse into the hemorrhages. The extraordinary acceleration of lipopigment formation from unsaturated fatty acids by hemoglobin is well known from in vitro experiments (Casselman 1951; Tappel 1955; Porta 1963). In this way, the formation of a lipopigment that is independent of cells could very well result. Extracellular lipochromes have occasionally been described in other organs, too (Györkey et al. 1968; Doyle et al. 1973), without, however, sufficient proof up to now that they are histochemically identical to hemoceroid. But even histologically, the occurrence of hemoceroid independent of macrophages inside larger hemorrhages is evident. Because the polymerization of unsaturated fatty acids can be increased as a result of the inhibition of oxidation (Wolman 1975), the presence of which can be assumed in this milieu, extensive utilization of reactive groups of the aliphatic molecules for polymer formation is conceivable.

Hence, the hemoceroid, apparently of extracellular origin, represents a particularly highly polymeric lipopigment with only a few oxidizable groups that is just secondarily phagocytosed. Consequently, not due to its composition alone, but probably also with regard to its genesis, it can be placed as the third chromolipid in the same category as the autophagously arising lipofuscin and the heterophagously formed ceroid.

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