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Timing of Cortical Contusion

Correlation Between Histomorphologic Alterations and Post-traumatic Interval

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Summary. One hundred and sixteen cases with known intervals between the time of brain injury and the time of death were systematically examined for selected histologic alterations in an attempt to facilitate the timing of histomorphologic alterations in cortical contusions following closed brain injury. The following criteria were considered: erythrocytes, polymorphonuclear leukocytes, scavenger cells, hemosiderophages, lymphocytes, hematoidin, degenerating neurons, neuronophagy, axonal swelling, protoplasmic astrocytes, piloid astrocytes, siderin-containing astrocytes, edema, increase of vessels, fibroblasts/fibrocytes, and collagenous fibers. The post-traumatic interval ranged between 0 min and 58 years.

Following routine staining, the paraffin sections were evaluated, and the presence of the selected histomorphologic criteria was determined (i.e., the time at which the criteria were demonstrable for the first and the last time during the post-traumatic interval in cases with different times of post-traumatic survival). The relative frequency of those cases with positive findings was calculated for each criterium in each observation period. The limits of confidence for the respective relative frequency was estimated with a statistical reliability of 95% according to Clopper and Pearson (1934) in the cases with demonstrated individual histomorphologic criteria. The distribution-free tolerance intervals with which each histomorphologic criterium is to be expected with 95% reliability within the observation period were established. The statistical data were compared with some of the data presented in the literature.

Key words: Brain injury, histomorphologic criteria – Cortical contusion, timing

Zusammenfassung. Um eine zeitliche Einordnung von histomorphologischen Veränderungen in Rindenprellungsherden nach gedecktem Schädel-Hirn-

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Trauma zu ermöglichen, wurden ausgewählte histologische Veränderungen an 116 Fällen mit bekanntem posttraumatischen Intervall systematisch untersucht. Berücksichtigt wurden folgende Kriterien: Erythrozyten, Granulozyten, Abräumzellen, Siderophagen, Lymphozyten, Hematoidin, Nervenzelldegeneration, Neuronophagie, Axonauftreibungen, protoplasmatische Astrozyten, piloide Astrozyten, Siderin-enthaltende Astrozyten, Ödemausbildung, Gefäßvermehrung, Fibroblasten/Fibrozyten und Kollagenfaserbildung. Das posttraumatische Intervall lag zwischen 0 min und 58 Jahren.

Bei der Auswertung wurden Paraffinschnitte nach Routinefärbungen daraufhin untersucht, ob die ausgesuchten histomorphologischen Kriterien vorhanden waren und wann sie während des posttraumatischen Intervalls jeweils erstmals und letztmals nachweisbar wurden. Die relative Häufigkeit der Fälle mit positivem Befund wurde für jedes Kriterium während der jeweiligen Beobachtungszeitspannen berechnet. Die Vertrauensgrenzen für die jeweilige relative Häufigkeit der Fälle mit Nachweis der einzelnen histomorphologischen Kriterien wurde mit einer statistischen Sicherheit von 95% nach Clopper und Pearson (1934) geschätzt. Die verteilungsfreie Toleranzgrenze, nach der mit 95%iger Sicherheit das jeweilige histomorphologische Kriterium innerhalb der Beobachtungszeitspanne zu erwarten ist, wurde berechnet. Die Einzeldaten wurden mit Daten im Schrifttum verglichen.

Schlüsselwörter: Schädel-Hirn-Trauma, histomorphologische Veränderungen – Rindenprellungsherde, Altersbestimmung

Many studies of neuropathologic alterations after craniocerebral damage and of pathologic alterations due to cortical contusions have been published. Some of these publications include information on pathomorphologic alterations during certain defined laps between injury and death (post-traumatic interval). Systematic studies of the timing of pathomorphologic alterations, however, are limited: cortical contusions and/or cerebral alterations due to trauma by G. Müller (1930), Krauland (1973), and Eisenmenger (1977); brain tissue damage due to anoxia by Spielmeyer (1922); Környey (1955), and Jacob (1961). This is surprising since the timing question is often important for medicolegal problems and for establishing the pathogenesis.

In this investigation cortical contusions of various post-traumatic intervals were studied to determine whether or not certain histomorphologic alterations were present. If the histomorphological criteria appear and disappear at regular intervals, the possibility of establishing their timing limits should be investigated. In this study, special emphasis was placed on the statistical evaluation of the material; some of the findings have already been published elsewhere (Oehmichen and Raff, 1978).

Material and Methods

1. Brain Tissue

Autopsy material from the Institute for Brain Research, University of Tübingen, was examined for the following criteria:

a) Closed craniocerebral damage, confirmed by anamnesis, without other demonstrable brain damage or post-traumatic epilepsy;

b) Known interval between damage and the time of death;

c) Macroscopically visible cortical contusions at the basal parts of the frontal and/or temporal lobes;

d) No clinical or morphologic indications of intravital interruption of intracerebral circulation for a longer period of time.

The actual cause of death was not considered in the selection of cases. A total of 116 cases fulfilled the criteria mentioned above.

2. Staining of Brain Tissue

After the formalin-fixed brain specimen was dissected into frontal sections, block-shaped tissue specimens were removed from the area of the cortical contusion. Tissue that was not macroscopically altered was also included. In accordance with routine methods, the tissue specimens were embedded in paraffin and cut into 7 to 10 µm-thick sections.

The following stains were included in the statistical evaluation: hematoxylin and eosin stain (H & E), Van Gieson stain, iron determination via the prussian blue reaction, cresyl violet stain. The following stains were also undertaken in special cases: demonstration of glial filaments with Holzer's stain, axonal stain according to Wölke, myelin stain according to Klüver and Barrera, Masson's trichromatic stain, PAS reaction according to Graumann (1953), and naphthol AS-D-chloroacetate esterase according to Leder (1964).

3. Histologic Evaluation

Only those sections were statistically evaluated which were stained according to the first group of methods mentioned. The following *histomorphologic criteria* were included, without reference to the quantity:

a) Reaction of blood cells via demonstration of erythrocytes, polymorphonuclear leukocytes, scavenger cells (i.e., monocytes, erythrocyte-, leukocyte-, or fat-containing macrophages), hemosiderophages (i.e., hemosiderin-containing macrophages), hematoidin (intracellular and extracellular).

b) Reaction of neurogenous tissue via demonstration of neuronal degeneration, neuronophagy, axonal swelling, protoplasmic alteration of astrocytes, piloid alteration of astrocytes, siderin-containing astrocytes.

c) Reaction of local mesenchymal tissue including edematous alterations (increased permeability of the vessels for blood plasma) via demonstration of increase of vessels, increase of fibroblasts and/or fibrocytes, increase of collagenous fibers.

Differentiation of the three reacting tissue types is schematic; the overlapping processes were not considered.

The differentiation was pragmatic. All selected histomorphologic criteria are ascertainable with the four routine stains. However, the additional stains mentioned above were carried out to confirm some of the findings if necessary. The findings were regarded as positive if the histomorphologic criteria appeared at least three times in each section (e.g., demonstration of three hemosiderophages). A clear-cut alteration in the stainability based on other histomorphologic criteria, such as edema or the demonstration of collagenous fibers, was always regarded as a positive finding.

The *morphology* of those histologic alterations is taken as basically established. Early demonstration of a *polymorphonuclear leukocyte reaction* was sometimes identifiable only through a discrete perivascular accumulation of neutrophilic granulocytes. *Hemosiderin,* as a product of blood decomposition in all short post-traumatic intervals of less than 2 months, was identifiable as fine granules and observed intracellularly. The granules became increasingly coarser as the survival period progressed; hemosiderin was also demonstrable extracellularly at this time.

All the various degenerative alterations of nerve cells were classified as *degenerating neurons* (i.e., shrunken hyperchromatic nerve cells in the vicinity of the damage and in the marginal

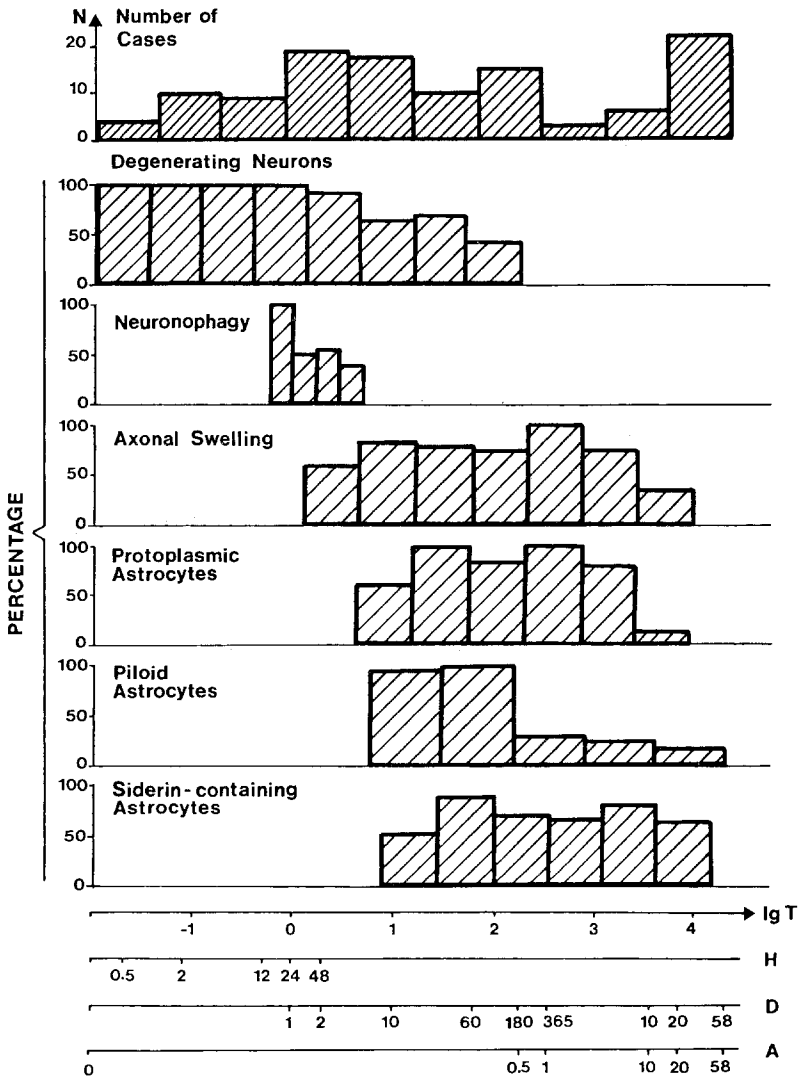

Diagram 1. Relative frequency of blood cell reaction in cortical contusions correlated with the post-traumatic interval. (Data expressed in percentage of cases [abscissa] with logarithmic representation of the time period [ordinate]. The various number of cases per group are derived from the total number of subjects exhibiting the histomorphologic criteria.) The total number of cases in correlation to the post-traumatic interval was demonstrated in the upper line of columns. H = hours; D = days; A = years

zone). Some cells were faded (pale) nerve cells. The Nissl substance was either clumped or marginally situated, close to the border of some cells; the cytoplasm contained areas of vesicular translucency. *Axonal swelling* was best demonstrated with the Van Gieson stain (and with Wölke's or Masson's stain); the axons appeared as round or oval platelets and/or bodies staining a homogeneous yellow. Differentiation from *protoplasmic astrocytes* almost never presented problems.

Only perifocal areas of translucency in the white matter and the cortex were considered as *edematous alterations,* which were characterized by pronounced pericellular and perivascular spaces caused by swelling of oligodendrocytes or of astrocyte footplates. Plasma exsudate into

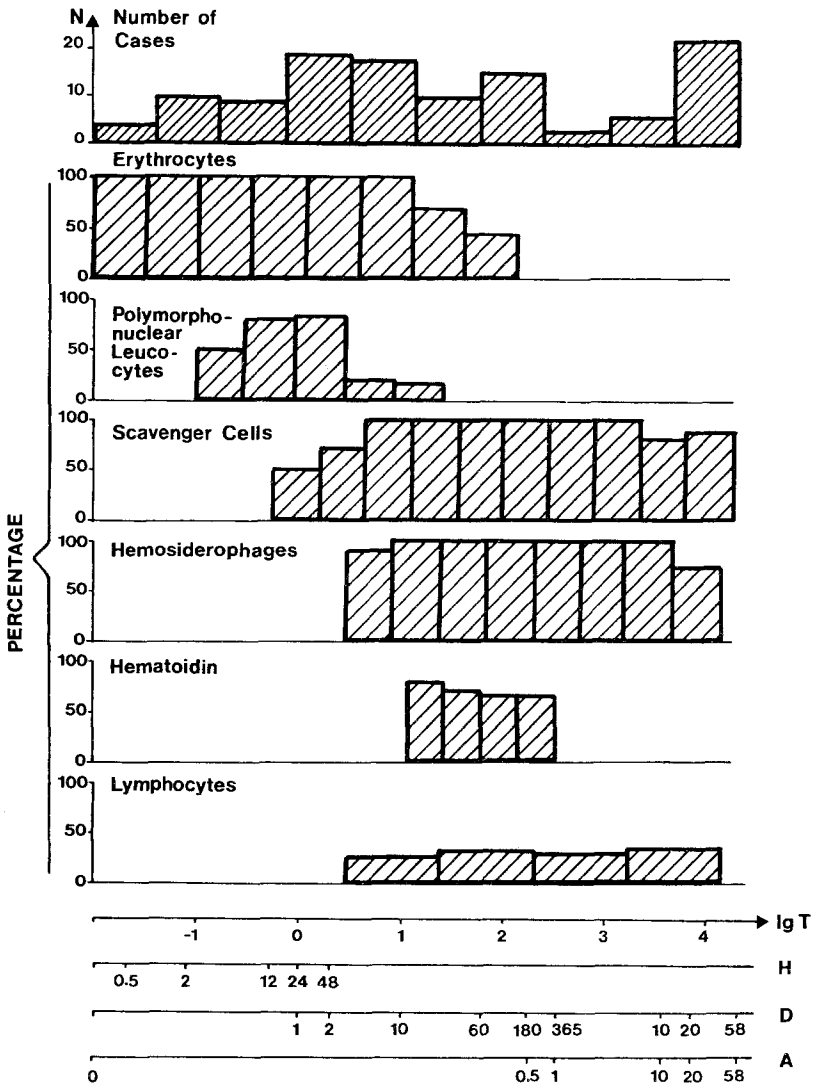

Diagram 2. Relative frequency of neurogenous tissue alterations in cortical contusions correlated with the post-traumatic interval (cf. also legend for Fig. 1)

the perivascular space around the damaged area was also included. However, such alterations were rare. On the other hand, the damage caused by the effects of edema, which understandably were present in all cases with a long interval between the traumatic event and the time of death, was not considered. The histologic limits are not clearly defined here and therefore sometimes the timing may be arbitrary.

4. Statistical Evaluation

Depending on the cases examined, the post-traumatic interval was recorded according to the duration in minutes, hours, days, or years. Additional evaluation of all material supplied the following information:

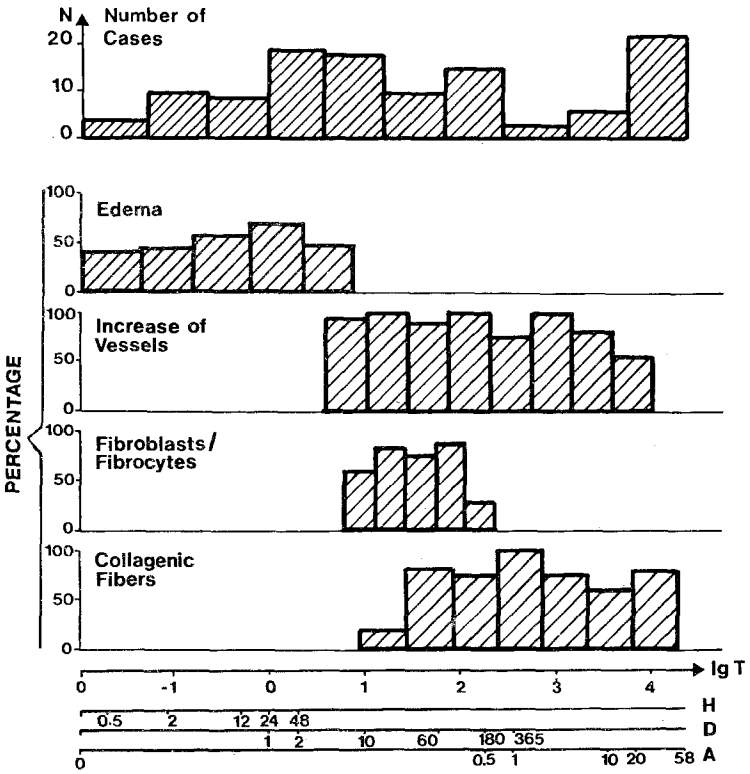

Diagram 3. Relative frequency of local mesenchymal tissue alterations including edema correlated with the post-traumatic interval (cf. also legend for Fig. 1)

a) First and last time during the post-traumatic interval at which a definite histomorphologic criterium was demonstrable *(observation period)*;

b) Frequency of a criterium in relation to the total number of cases studied within this observation period *(relative frequency)*;

c) Estimation of the *limits of confidence* for an observed relative frequency according to Clopper and Pearson (1934): a 95% statistical reliability for the limits of confidence for the relative frequency;

d) *Distribution-free (independent) tolerance intervals* (Sachs, 1978) according to which certain criteria appeared within the respective observation period with 95% reliability.

Results

The distribution of cases with different *post-traumatic intervals* in the examined 116 cases may be seen in the upper portions of Diagrams 1–3; the data is given in semilogarithmic form. The number of subjects surviving the trauma for a short period of time (not longer than 2 months) was considerably higher than that of subjects surviving for longer periods of time (up to 58 years). It may be assumed that the death of the subjects with shorter survival periods was directly or indirectly related to the effects of the trauma; the actual cause of death in some cases, however, was unrelated to the brain damage. As the interval between the

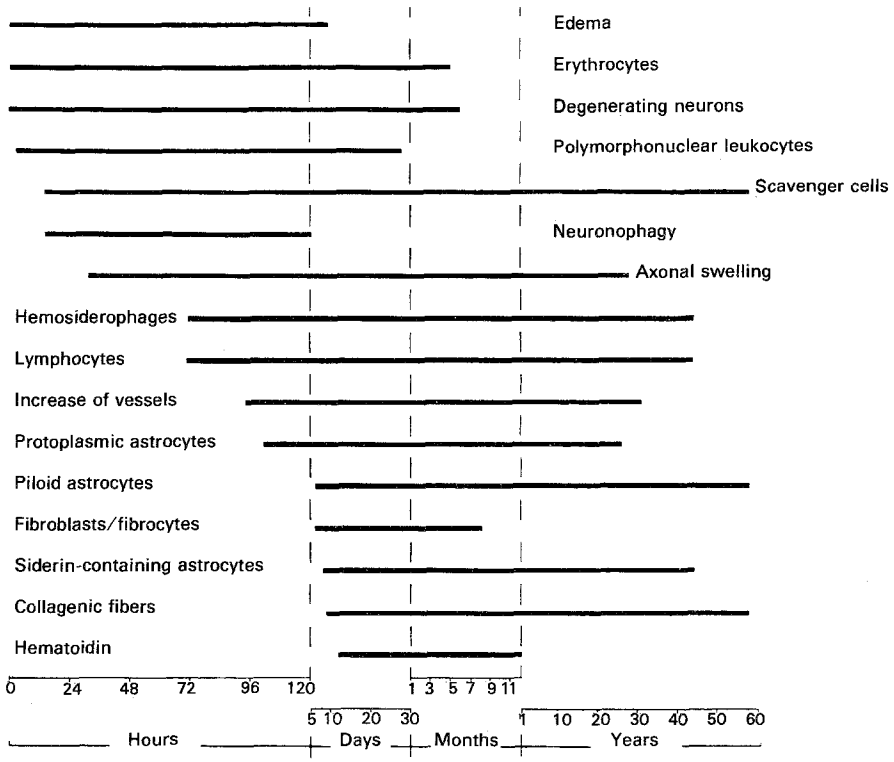

Diagram 4. Demonstration of post-traumatic periods during which the individual histomorphologic criteria were observed in our body of material

trauma and the time of death increases, the number of subjects dying from the effects of the trauma dropped. When the post-traumatic interval was longer than 6 months, death was caused by diseases or disorders unrelated to the trauma.

The bar charts in Diagrams 1–3 show the *frequency with which the histomorphologic alterations were observed* at certain time periods during the post-traumatic interval. The histomorphologic criteria presented in Diagram 1 were established on the basis of the blood cell reaction. In Diagram 2, the alterations of the neurogenous elements were demonstrated. Diagram 3 shows the mode of reaction for local mesenchymal tissue.

Some alterations were demonstrable in all cases examined in the respective time period at specific times after the trauma (e.g., erythrocytes, scavenger cells, degenerating neurons). Other alterations, however, should be regarded as facultative, e.g., the development of lymphocytic infiltrate or the formation of hematoidin.

Apparently, a correlation exists between the number of subjects with the respective histomorphologic criterium and the length of the period between the trauma and the time of death. This includes the initial appearance and disappearance of individual criteria as well as the distribution of the cases exhibiting the histomorphologic criteria during the observation period (Diagram 4).

Table 1. Observation periods for those histomorphologic criteria in cortical contusions considered criteria were observed in the cortical contusion (column 5). The limits of confidence for the respective with a 95% statistical reliability according to Clopper and Pearson (column 6). Calculation of expected with 95% reliability for the survival periods within the observation period after a cortical

Histomorphologic alterations	Demonstration time correlated with the time of the traumatic event (observation period)		Total number of examined cases during the observation period (*n*)
	First appearance	Last appearance	
Column	1	2	3
Erythrocytes	0	5 mo	81
Polymorphonuclear leukocytes	130 min	28 d	60
Scavenger cells	14 h	58 yr	97
Hemosiderophages	71 h	44 yr	74
Hematoidin	12 d	12 mo	29
Lymphocytes	71 h	44 yr	74
Degenerating neurons	0	6 mo	82
Neuronophagy	14 h	5 d	26
Axonal swelling	31 h	28 yr	81
Protoplasmic astrocytes	101 h	26 yr	63
Piloid astrocytes	6 d	58 yr	71
Siderin-containing astrocytes	8 d	44 yr	65
Edema	0	9 d	57
Increase of vessels	94 h	31 yr	72
Fibroblasts/fibrocytes	6 d	8 mo	40
Collagen fibers	9 d	58 yr	65

All other recorded data are presented in Table 1. The investigations and statistical computations were carried out to determine the probability for histomorphologic alterations in certain time periods. The *observation period* (Table 1, columns 1 and 2) indicates the time at which we observed certain criteria for the first and the last time. The *relative frequency* (Table 1, column 5) refers to all cases within this time period. The *estimated limits of confidence* for this observed relative frequency (Table 1, column 6) were stated with 95% reliability for every type of histologic criteria included here: the limits of confidence for three histomorphologic criteria were less than 20% (erythrocytes, 17%; scavenger cells, 14%; hemosiderophages, 13%); only three criteria, over 30% (hematoidin, 35%; neuronophagy, 40%; fibroblasts, 34%). The projected percentage of cases in each time period with a 95% reliability is expressed in terms of the *distribution-free tolerance interval* (Table 1, column 7). Only two histomorphologic criteria showed a distribution-free tolerance interval of less than 80% (hematoidin, 79%; neuronophagy, 72%); seven additional criteria were less than 90%; the remaining seven criteria were observed with distribution-free tolerance intervals of 90% or more.

The observation periods for the individual histomorphologic criteria are presented graphically in Diagram 4. The criteria are organized according to their

here (columns 1+2). Relative frequency of cases examined in which the respective histomorphologic relative frequency of those cases demonstrating the individual histomorphologic criteria—estimated the distribution-free tolerance interval with which the respective histomorphologic criteria may be contusion (column 7)

Number of cases with histomorphological alterations (observations)	Relative frequency of the observations (data expressed in percentages)	Estimated limits of confidence (Clopper and Pearsson, 1934) (data expressed in percentages)	Distribution-free tolerance intervals with 95% reliability (data expressed in percentages)
4	5	6	7
72	88.9	78–95	93
29	48.3	34–64	85
87	89.7	82–96	94
68	91.9	84–97	93
21	72.4	53–88	79
22	29.7	19–42	80
65	79.3	69–89	93
15	57.7	35–75	72
55	67.9	57–79	91
42	66.7	55–80	89
36	50.7	37–62	87
44	67.7	55–77	90
30	52.6	38–65	85
59	81.9	71–91	92
26	65.0	47–81	83
43	66.2	55–80	89

initial demonstration and their correlation with the period of time after the trauma. This diagram clearly shows the correlation between the appearance and disappearance of individual criteria and the length of the post-traumatic interval.

Rare histologic alterations, such as the demonstration of coagulation necrosis and ferrugination of nerve cells, were not included in the statistical evaluation. In 10 cases, *coagulation necrosis* was observed as early as 23 days and as late as 53 days after the trauma. *Ferrugination of nerve cells* was observed in 11 cases; the briefest post-traumatic interval was 228 h (9–10 days); the longest, 28 years.

Discussion

The timing of the *course of the reactive-inflammatory process* during the post-traumatic phase is partly reconstructable on the basis of the time sequence of the histomorphologic criteria which are individually recorded and evaluated. The traumatic event tears the vessels and, secondary, nerve tissue, and blood is exuded. The blood cells are located primarily perivascularly; blood plasma is exuded into

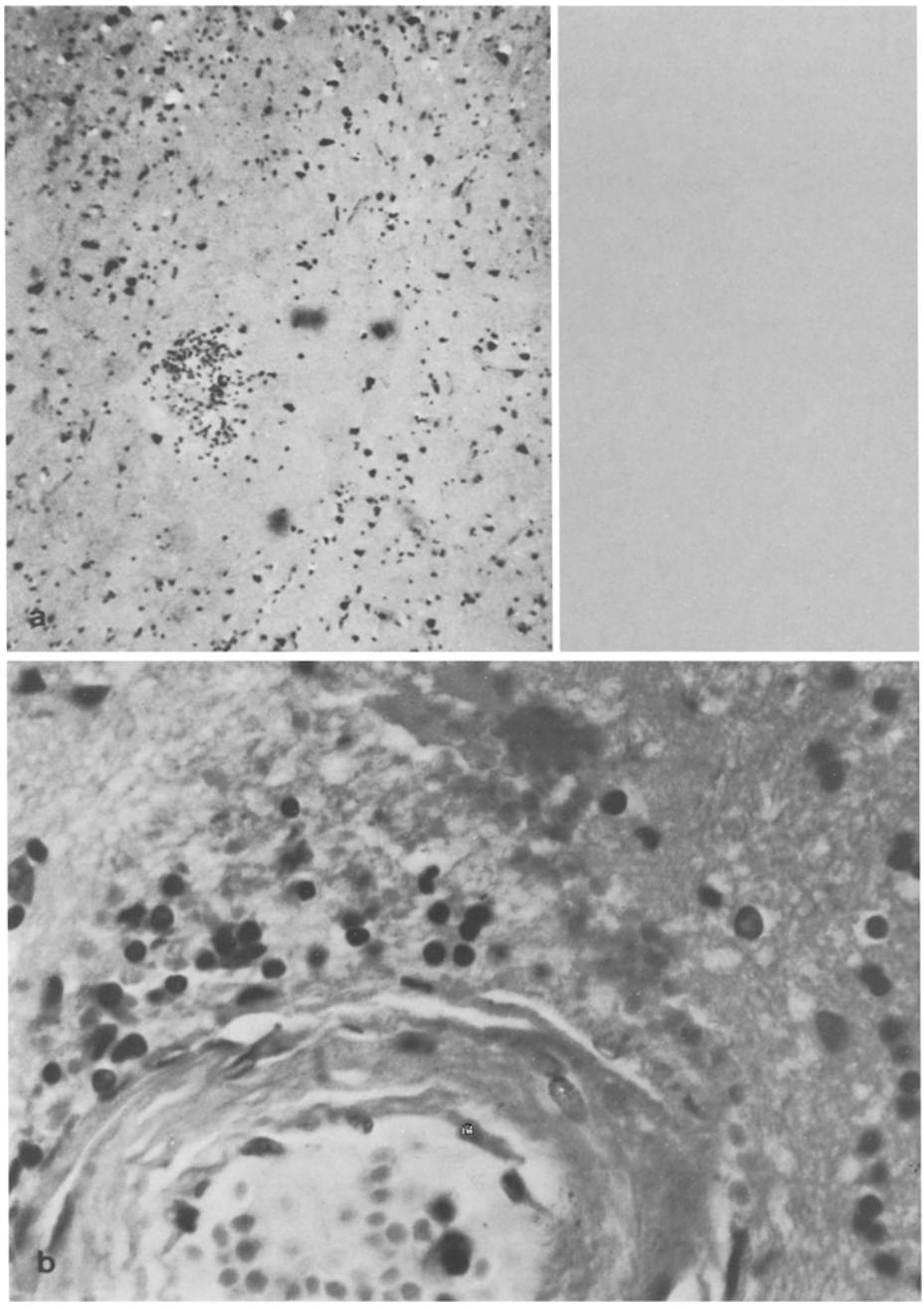

Fig. 1. Blood cell reaction in cortical contusions (survival time: 14 h): **a** polymorphonuclear leukocytes (H & E, ×40); **b** scavenger cells (extravasal monocytes) (H & E, ×500)

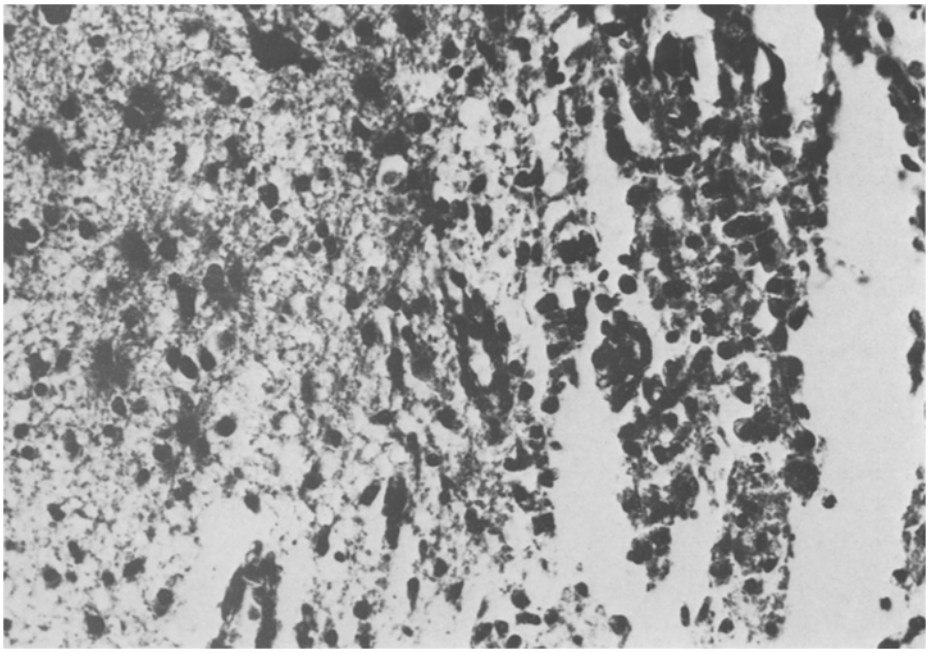

Fig. 2. Protoplasmic (reactive) astrocytes and scavenger cells in cortical contusion (survival time: 9 days); Van Gieson stain, × 500

regions of the brain some distance away, where it gives the appearance of edema. The surrounding nerve cells are damaged by the traumatic event and/or edema. This damage was demonstrable in all our cases, even when death occurred immediately after the traumatic event.

There are two types of response to these alterations: A blood cell reaction facilitating the removal of necrotic tissue which is produced when the blood cells are exuded (polymorphonuclear leukocytes [Fig. 1a]; scavenger reaction with monocytes [Fig. 1b]; erythrocyte-, fat-, leukocyte-, hemosiderin-, or hematoidin-containing macrophages) and a local neurogenous tissue reaction in the form of axonal swelling, neuronophagy, formation of protoplasmic astrocytes (Fig. 2) which additionally cleanse the local tissue of foreign matter particles (damaged neurons, edema fluid containing ions and proteins). The length of these acute-subacute reactive phases will depend on the extent of the damage and the concomitant alterations, particularly extracerebral disorders.

The beginning of the phase of the local mesenchyme reaction (the end stage, cf. Peters, 1955) usually occurs around the 4th to the 6th day after the trauma; it is basically unrelated to the duration of the mentioned reactive alterations. This phase is characterized by fibroblasts, increase in capillaries (Fig. 3) and glial filaments (piloid astrocytes) (Fig. 4), as well as the formation of collagenous fibers. The residue from this stage and the individual alterations of the reactive phase (e.g., siderophages) have been identified in varying degrees decades after the traumatic event.

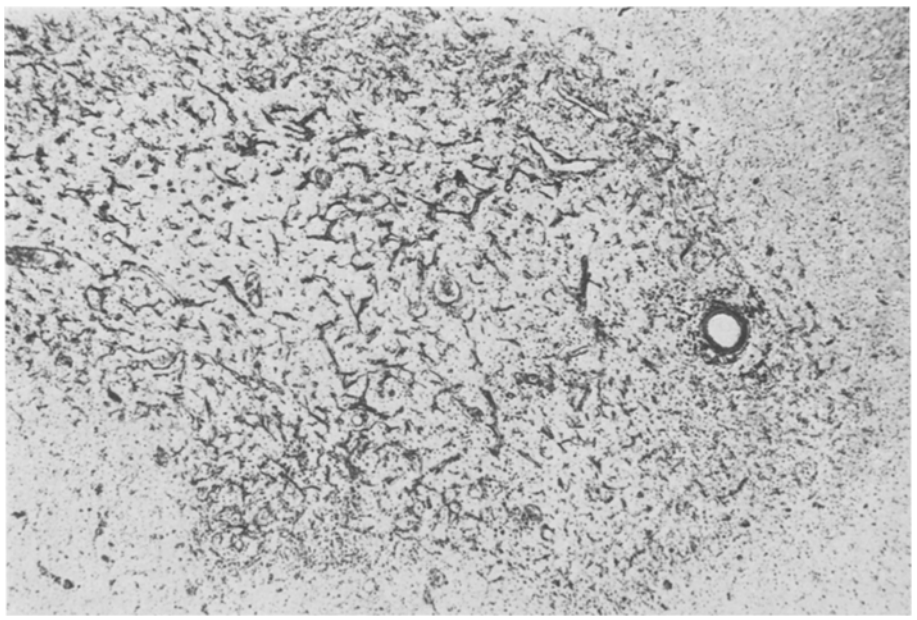

Fig. 3. Reactive proliferation of capillaries in cortical contusion (survival time: 21 days); Van Gieson stain, × 160

Apart from the above-mentioned investigations on time-dependent histomorphological alterations in cortical contusions after craniocerebral damage, many studies on the initial appearance of individual histomorphologic alterations have been published. These *observations of other authors* correspond basically with our findings. Only a brief presentation is provided below since a comprehensive survey would go beyond the scope of this study.

Erythrocytes appear in damaged brain tissue immediately after the trauma-related tearing of the vessel. Strikingly erythrocytes are observed as long as 5 months after the trauma. Peters (1955; Esser, 1931) assumed that repeated diapedetic hemorrhages occur after the trauma which very probably are produced by secondary circulatory disturbances; we would tend to agree with him.

Peters (1955) reported the earliest form of a reactive alteration to be a *polymorphonuclear leukocyte* reaction. He observed an increased number of these cells after 2 h. In the literature, the *scavenger cell* reaction was described as a reaction of microglia, fat granule cells, monocytes, and macrophages; all cells which are also classified as mononuclear phagocytes (Oehmichen, 1978). Carmichael (1929) observed them as early as 6 h after brain damage; Rio Hortega (1919) after 12 h; Jacob (1961) after 14 h; Nevin (1967), Masuda (1969) and Penfield (1932) after 24 h; Macklin and Macklin (1920) and Lindenberg and Freytag (1957) after 48 h. An increase in free subarachnoid phagocytes was observed in the course of the cytologic examination of cerebral spinal fluid as early as 12 h after traumatic damage (Oehmichen, 1976). Krauland (1973) reported scavenger cells in the brain damage region several years after the traumatic event.

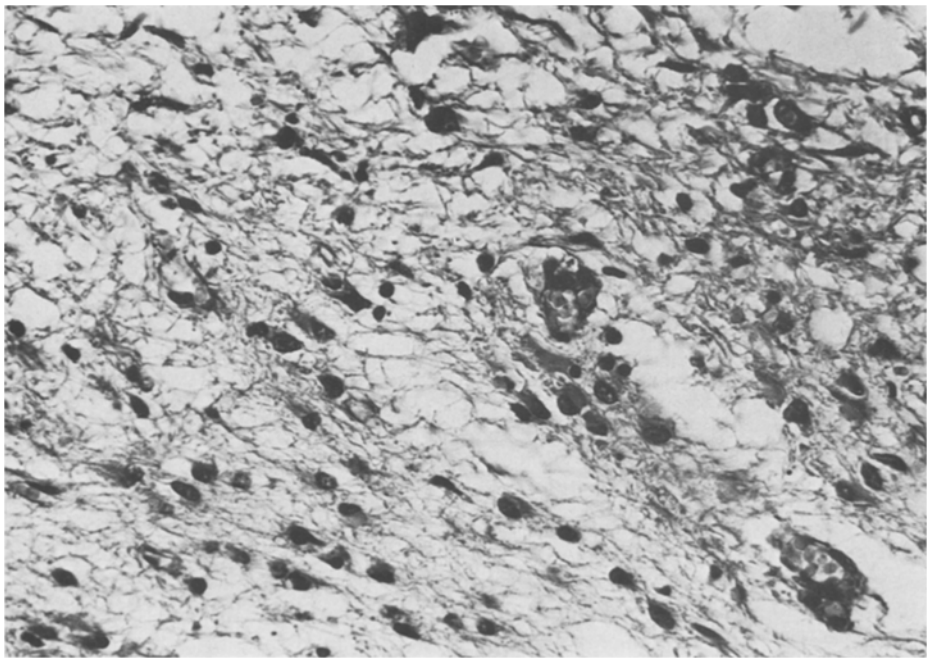

Fig. 4. Increase of piloid (fibrocytic) astrocytes during the last phase of reaction (survival time: 4 weeks); Van Gieson stain, ×500

The clear-cut demonstration of phagocytosis via mononuclear phagocytes is best accomplished by demonstrating an *erythrocytophagocytosis.* These alterations were not considered in our evaluation, because the sections were too thick for a detailed study. We found them in some cases (Kennady, 1967), but never earlier than 8 h after the trauma.

The destruction of erythrocytes to *hemosiderin* only occurs intracellularly. Siderin-containing macrophages were initially observed at various times after intracerebral hemorrhages: 6 days (Strassmann, 1949), 5 days (Baggenstoss et al., 1943; Wieczorek, 1964; Krauland, 1973), 4 days (Rautenbach, 1968), 3 days (Hallermann and Illichmann-Christ, 1973), 48 h with mice (Strassmann, 1949) and dogs (Krauland, 1973). The hemosiderin is first amorphic: It becomes increasingly crystallized and finally is mineralized (Schwietzer, 1953). In its mineralized state, siderin can no longer be mobilized and therefore may remain dormant for decades.

Hematoidin is crystallized bilirubin; it may also be formed extracellularly. Strassmann (1949) observed hematoidin 10 days after a hemorrhage in two cases; we observed it for the first time after 12 days. In contrast to the long period of demonstration for siderin, hematoidin was not observed after a post-traumatic interval of more than 12 months. Crystallized hematoidin is reabsorbed relatively quickly (Howard and Cooper, 1955). Hematoidin, however, can be found as long as 8 years after the hemorrhagic event (Boellaard, 1977) if the hematoidin is encapsulated in a thick connective tissue membrane.

The appearance of *degenerative alterations of neurons* immediately after death was questioned by Courville (1964); other authors, however, described these alterations (Peters, 1955; Eisenmenger et al., 1978). Peters suggested that acidification of the tissue was rapid. Such alterations may be similar to alterations caused by postmortal brain damage (Cammermeyer, 1972). Other authors (Colmant, 1965) observed distinct neuronal alterations due to ischemic damage after 20 to 30 min, at the earliest.

Schröder and Wechsler (1965) observed pronounced *axonal swelling* 3 to 5 days after the trauma. After 12 h, they noted progressive axonal alterations. Kreutzberg and Peters (1965) observed axonal alterations in animal experiments as early as 6 h and Colmant (1965), 10 to 20 h after the trauma.

Colmant (1962) observed *protoplasmic astrocytes* for the first time 24 h after brain damage. Using histochemical methods, reactive astrocyte alterations were demonstrated earlier (6—10 h: Mossakowski and Penar, 1972; 12 h: Rubinstein et al., 1962). Distinct protoplasmic astrocytes were observed for the first time relatively late with our method.

Peters (1955) found no indication of edema with subjects dying immediately after the injury. We, however, observed beginning edematous alterations in all such cases. These findings also correspond to the findings of Schröder and Wechsler (1965): signs of edema observed 15 min after the trauma; pronounced edema, 3 to 5 days later. Edema was confirmed with computer tomography 20 min after the trauma (Kobrine et al., 1977). According to the authors, demarcation with lacunal fields was first discernible after 10 to 12 h (Lindenberg and Freytag, 1958; Sellier and Unterharnscheidt, 1963).

The beginning of progressive alterations (e.g., *increase in capillaries, fibroblasts, and collagenous fibers*) were observed by all authors between the 4th and 6th day after the trauma (Peters, 1955; Lindenberg, 1957; Sellier and Unterharnscheidt, 1963; Eisenmenger, 1977).

By way of a *criticism of our methods,* it should be noted that no quantification was udertaken in the material examined; demonstration of the individual observations was interpreted as positive. Since no serial sections were examined, it is possible that false-negative results were obtained in some cases. False-positive results, however, were not obtained.

The following factors which very probably influence the rate of one part of the reactive processes should also be mentioned (cf. Müller, 1930). We did not consider any additional effects of damage to the brain and other organs or the age of the subject at the time the injury was inflicted. As mentioned previously, the cause of death was not taken into consideration. This factor may well have been influencial in cases with a relatively short survival period. At last, sometimes individual criteria are not clearly distinguishable from normal findings. As a result, errors are unavoidable.

Within these limits, two *conclusions* may be drawn from our observations: *First,* each individual histomorphologic criterion was always identifiable in the posttraumatic interval during clearly defined time periods. The appearance and disappearance may be considered "regular." Overlapping of the timing for individual histomorphologic criteria was observed. If the age of a cortical contusion is to be determined on the basis of histomorphologic criteria, the timing may be

established on the basis of the criteria presented in Fig. 4. The possibilities for differentiating the various times is limited. A long-term study can and would provide a general survey of the possibilities for temporal differentiation using routine methods. Because of the methodological limitations, longer periods of time were not differentiated during the post-traumatic interval. The histochemical methods also considered by Eisenmenger (1977) provided no other important criteria for the timing. Additional criteria may possibly be established by using other staining methods and/or through electron-optic studies.

Second, the statistical evaluation of our data made it possible to establish the probability with which individual histomorphologic criteria may be expected within any observation period (the so-called distribution-free tolerance interval). In our material, the probability depends on the number of individual observations during any one time period. The probability is low (less than 70%) with the facultative histomorphologic criteria, such as hematoidin, lymphocytes, and neuronophagy. All other criteria demonstrate a distributions-free tolerance interval of more than 80%.

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