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Pulmonary Histopathology and Survival Period in Morphine-Involved Deaths

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ABSTRACT: For an evaluation of the survival period in morphine-involved deaths, changes of pulmonary histopathology were investigated in a total of 90 morphine-associated fatalities. Although pulmonary histopathology proved to be heterogeneous, several distinctive histological patterns emerged. While the subgroup with short courses of intoxication (<1 h, $n = 15$) was mostly characterized by slight/moderate alveolar edema (12/15), severe hemorrhages (12/15) and marked acute emphysema (9/15), the phenomena of massive edema (8/15), missing/slight hemorrhages (8/15) and absent/slight emphysema (11/15) dominated in the group with intermediate survival times (1–24 h, $n = 15$). Intravascular leukocyte accumulations (shock equivalents) occurred in the first group only once, but in the group with the longer survival time in 10 of 15 cases. Delayed deaths (>24 h, $n = 4$) were mainly characterized by purulent bronchitis/pneumonia. Those fatalities ($n = 56$) that could not be classified by anamnestic data were assessed by histological criteria. In comparison with the evaluation of the survival period by toxicological analyses, concordance was found in 46 cases. Pulmonary histopathology is not a tool for an exact graduation of survival time, but the combination of several key parameters can provide criteria for a differentiation between short (<1 h) and longer courses of intoxication.

KEYWORDS: forensic science, forensic pathology, forensic toxicology, pulmonary histopathology, survival period, morphine-involved deaths, drug abuse, morphine

The evaluation of the survival time following fatal intravenous injection of opiates can become relevant to various issues in forensic casework. In particular, when failure to give assistance—for example, common usage of heroin by several persons, death of one participant—or possible drug administration by others come into question, the time interval between injection and death is of interest. In practice, toxicological parameters and their kinetics are mainly used for this purpose. Thus, Spiehler (1,2) introduced a computer-assisted interpretation of toxicological data in morphine-involved deaths: blood unconjugated morphine, percent unconjugated morphine in blood, and brain total morphine were most useful in estimating time of death since drug injection. In addition, microscopic indicators such as wound age estimation at the injection site and pulmonary histomorphology can be taken into consideration. The latter has been investigated by Siegel et al. (3–5). According to the survival period after heroin injection four subgroups with different changes in pulmonary histopathology were established (Table 1) (3). On the one hand, Siegel et al. made very distinct statements resulting in the graduation as shown in Table 1; on the other hand, temporal overlappings and gaps occur and the histological parameters were not defined clearly.

Bearing in mind that time-dependent histopathological alterations after an event (beginning of an intoxication) also involve the lung as a shock organ, we investigated a total of 90 drug-induced fatalities within the framework of pulmonary "histokinetics."

Material and Methods

The study group included 90 morphine-involved fatalities (Table 2). In all cases opiates, heroin metabolites and/or morphine, were detectable, partly in combinations with alcohol or psychopharmacological agents. In 34 cases, the survival time after injection of drugs was documented (judicial inquiry, testimonies). Based on the accuracy of these anamnestic data and the histological findings presented as follows, three subgroups were formed: cases with "short" (<1 h), "intermediate" (1–24 h) and "long" survival periods (>24 h). The results were compared with the survival times due to the toxicological analyses. Unconjugated and conjugated morphine were measured in blood, lung, liver, brain and—if available—urine by gas chromatography/mass spectrometry (GC/MS) (6). The data were interpreted according to the model of Spiehler (1) (survival periods of <3 h, 3–12 h, >12 h). This was completed by the

TABLE 1—*Pulmonary histopathology and survival period in morphine-involved deaths (according to Siegel et al. (3)).*

Stage	Survival Period	Pulmonary histopathology
1	Minutes to 3 hours	Congestion, edema; foci of slight atelectasis/emphysema; a few alveolar macrophages
2	3–7 hours	Congestion, edema; foci of slight atelectasis/emphysema; many macrophages; some red cells, very few granulocytes
3	5–12 hours	Congestion, edema; alveolar hemorrhages; many macrophages; exudation of granulocytes; occasionally hyaline membranes
4	>24 hours	Acute exudative lobular pneumonia; other changes as described above

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TABLE 2—*Material: composition of study group.*

Study group: 90 morphine-involved fatalities (♂ 77, ♀ 13, mean age: 26.9)	
Survival period documented	
• short (<1 h)	(n = 15)
• intermediate (1–24 h)	(n = 15)
• long (>24 h)	(n = 4)
• not precisely defined	(n = 56)
Survival period due to toxicological findings (according to Spiehler, 1989)	
• <3 h	(n = 42)
• 3–12 h	(n = 31)
• >12 h	(n = 7)
• not exactly evaluated	(n = 10)

detection of 6-monoacetylmorphine (6-MAM) in brain and urine (6).

In the histologic investigations at least five specimens per case from different lobes of the lung were fixed in 4% formalin and embedded in paraffin. Sections of 2–3 μm thickness were prepared and stained with H&E, PAS, EvG and—if necessary—naphthol-AS-D-Cl-acetate-esterase. Microscopic evaluation followed without previous knowledge of the survival period with regard to general morphological changes of the pulmonary structure (edema, hyperemia, alveolar hemorrhages, focal emphysema, local atelectasis) and, in particular, alterations of blood vessel contents. Intravascular cell accumulations were regarded as positive, if at least 20% of the cells in a vessel were nonerythrocytic. Both measurements were graduated in a semiquantitative way in relation to the total of alveoli (<10%/10–50%/>50% or graduation in absent—slight/moderate—strong/marked) or vessels (<5%/5–20%/20–50%/>50%). In addition, the presence of inflammatory lesions was noted (granulocytes and lymphocytes: intra-alveolar, interstitial, bronchiolar, peribronchiolar, perivascular).

Those 56 cases without anamnestic data on survival periods were evaluated by histological parameters. The results were compared with the intervals of survival found by using toxicological methods.

Results

Pulmonary Histopathology in Relation to the Survival Period

The evaluation of results showed that three ranges of the survival period after consumption of opiates could be distinguished (<1 h, 1–24 h, >24 h; Table 2). The corresponding pulmonary histopathology was altogether heterogeneous, but exhibited several different basic patterns. The parameters of edema, alveolar hemorrhages and focal emphysema were individually non-specific; in combination, however, they proved to be "key parameters" for a temporal differentiation. The threshold values for missing/slight or moderate and strong/marked expression of these histological indicators were involvement of 10% (hemorrhages, emphysema) and 50% (edema) of all alveoli. Apart from these general changes of lung structure the phenomenon of intravascular leukocyte accumulations had a high discriminatory function as to the survival period (see the following). By contrast, the parameters of hyperemia and local dystelectasis were present almost invariably and therefore not included in the final evaluation.

The results of microscopic examinations in those 34 cases with definite survival times are summarized numerically in Table 3. The three subgroups are to be characterized in brief form as follows:

Short Survival Periods (<1 h)—This group was characterized by a rather slight or moderate alveolar edema (12/15), but revealed severe perivascular and intra-alveolar hemorrhages (12/15) (Fig. 1) and frequently a marked acute focal emphysema (Fig. 2). Intravascular cell accumulations occurred only once. Acute inflammatory alterations (purulent bronchitis/pneumonia) were not observed.

Intermediate Survival Periods (1–24 h)—In this subgroup with longer courses of intoxication—a further differentiation within the 1–24 h interval was not possible by the methods applied—the phenomena of strong lung edema involving more than 50% of all alveoli (Fig. 3), missing or slight hemorrhages and absent or little focal emphysema dominated. Hemorrhages in individual cases, however, showed leukocyte reaction pointing to a longer survival. Further, the shock equivalent intravascular cell accumulations

TABLE 3—*Synopsis of histological pulmonary changes in different survival periods (own results).*

Phenomenon	Survival time	Short <1 h (n = 15)	Intermediate 1–24 h (n = 15)	Long >24 h (n = 4)
Edema	Slight/moderate (≤50%)	**12**	7	2
	marked (>50%)	3	**8**	2
Alveolar hemorrhages	Absent/slight (<10%)	3	**8**	2
	marked (≥10%)	**12**	7	2
Focal emphysema	Absent/slight (<10%)	6	**11**	3
	marked (≥10%)	**9**	4	1
Intravascular cell accumulations		1	**10**	**1**
Purulent bronchitis/lobular pneumonia		0	7	**3**
Lung weights (in g)		1688	1678	2028

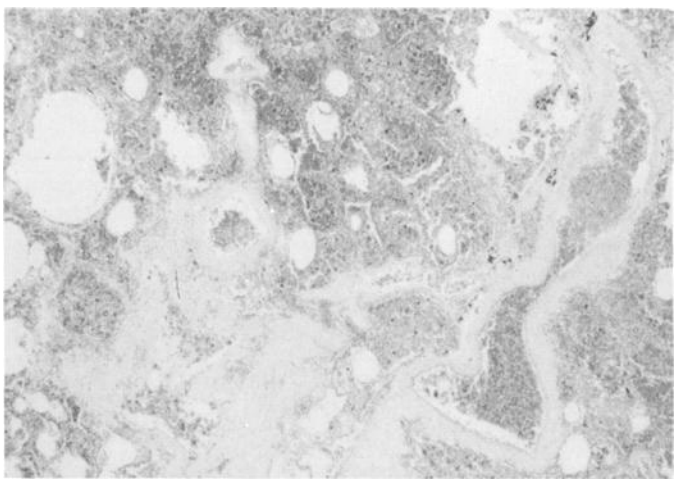

FIG. 1—*Case with short survival time (<1 h): extensive perivascular hemorrhages, H&E, original magnification ×50.*

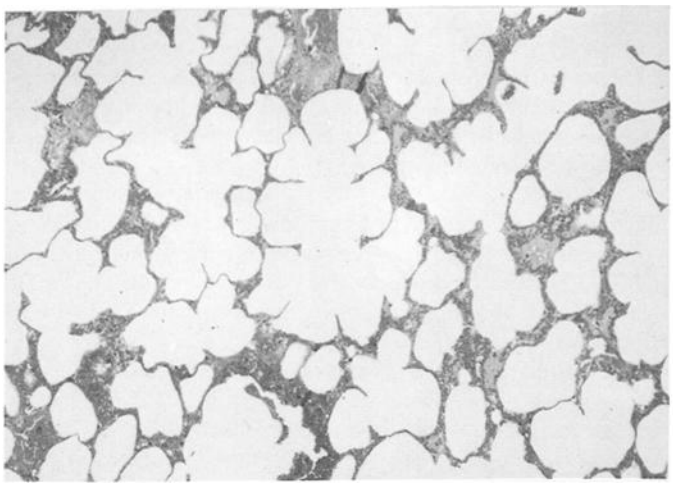

FIG. 2—*Case with short survival time (<1 h): marked focal emphysema, H&E, original magnification ×50.*

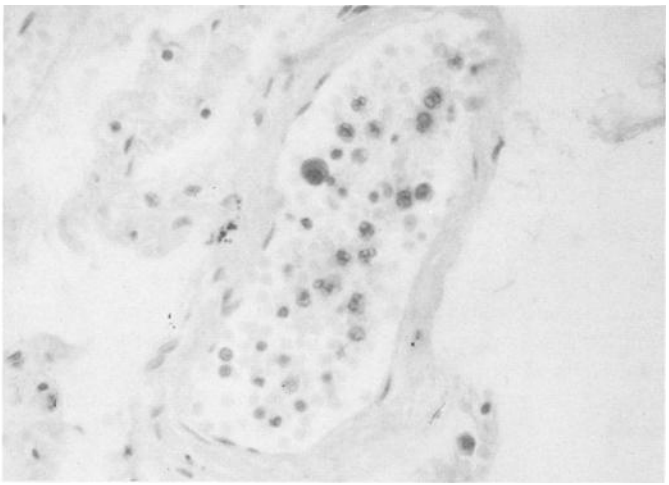

FIG. 4—*Intermediate survival periods (1–24 h): disseminated intravascular cell accumulations, naphthol-AS-D-Cl-acetate-esterase, original magnification ×500.*

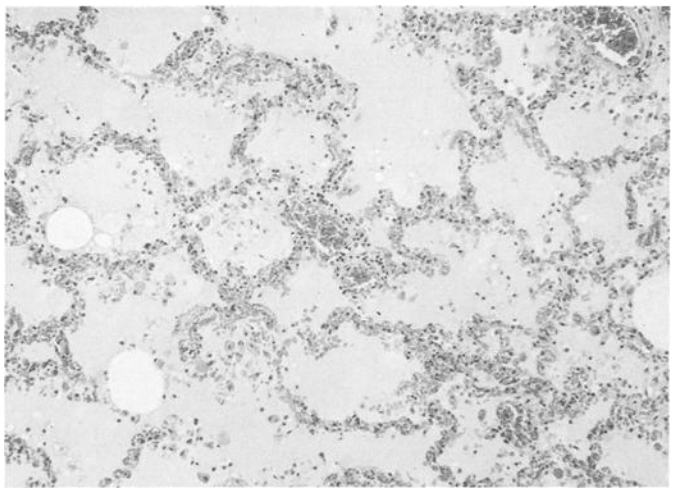

FIG. 3—*Case with intermediate survival time (1–24 h): severe intraalveolar edema with concomitant congestion, H&E, original magnification ×125.*

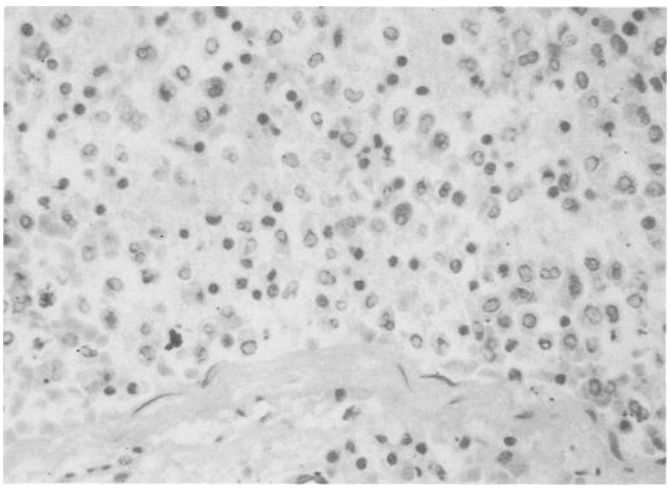

FIG. 5—*Intermediate survival periods (1–24 h): detail from aggregated intravascular cell accumulations, H&E, original magnification ×500.*

proved to be an essential marker for cases with longer (>1 h) survival times. They appeared in 10 of 15 cases. These accumulations occurred partly as aggregates filling the entire vessel and partly in a disseminated manner (Figs. 4 and 5). They mainly involved medium-sized and smaller arteries and consisted of polymorphonuclear and juvenile granulocytes, lymphocytes, monocytes and some obviously immature bone marrow cells. About one-half to two-thirds of the cells gave positive stains with naphthol-AS-D-Cl-acetate-esterase, and thus belong to the myeloic system. On the whole these changes were mainly restricted to a few sections of a case or even to a few vessels within one section. In most cases only several pulmonary vessels (<20%) showed intravascular cell accumulations. Moreover, in this subgroup inflammatory lesions, mainly of minor degree, gained in importance and were detectable in one-half of the cases (7/15).

Long Survival Periods (>24 h)—Delayed deaths (1–5 days) were mainly characterized by purulent bronchitis and marked pneumonia (Fig. 6).

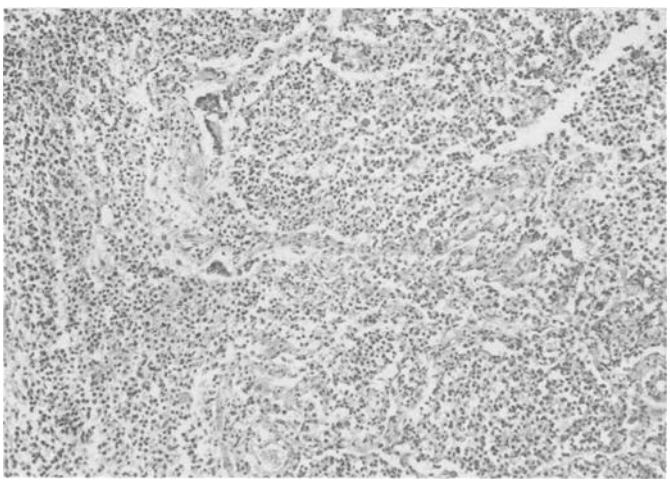

FIG. 6—*Case with long survival time (>24 h): severe purulent bronchitis and pneumonia, H&E, original magnification ×125.*

The additionally registered mean combined lung weights are shown in Table 3.

Comparison of Histological and Toxicological Methods in Evaluating the Survival Period

In a second step we investigated whether pulmonary histopathology can confirm and extend the toxicological findings and if it can be used for an evaluation of the survival time in those 56 cases without anamnestic data. In this connection the value of the toxicological parameters is of interest. The evaluation of 18 cases with certain short and long survival times revealed (Fig. 7), that the toxicological interpretation of the survival period according to Spiehler (1) was correct in 15 cases (marked by horizontal lines). However, in three cases the toxicologic data were not consistent with the actual survival time (depicted by oblique lines).

When the survival period was assessed with both toxicological and histological methods in those cases, which could not be classified definitely by anamnestic data, concordance was found in 46 of 56 cases (toxicological 1-hour-interval based on a limiting value of >100 μg/kg 6-MAM in brain). Differences were observed in ten cases (Table 4).

Discussion

The occurrence of various acute and chronic alterations of organs and tissues in drug addicts, in particular after longer intravenous abuse of heroin, is well-known. Apart from local reactions at the site of injection there are well-recognized diseases of the heart, liver and lung (3–5,7–15). Cardiac pathology comprises acute inflammatory lesions (myocarditis, endocarditis) and chronic changes in the form of myofibrillar degeneration (stromal condensation, focal fibrosis, and mononuclear infiltration of the conduction system) (8,9,11,15). Hepatic lesions include nonspecific reactive hepatitis, chronic aggressive and acute viral hepatitis and cirrhosis (8–10,14); in most studies less than 10% of all cases revealed a normal liver histology.

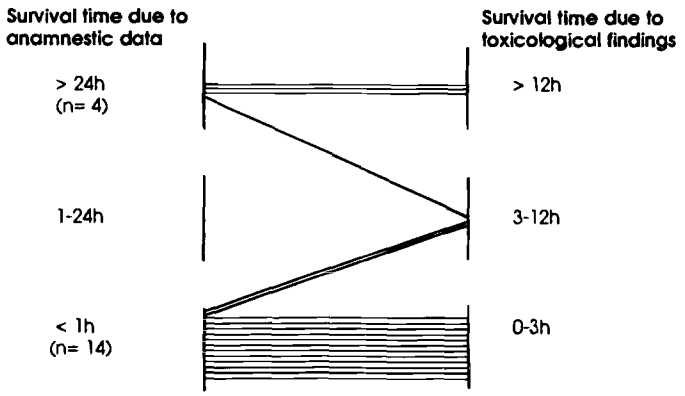

FIG. 7—*Value of toxicological findings, comparison with anamnestic data (each line represents one case).*

TABLE 4—*Differences in the evaluation of the survival time between toxicology and pulmonary histopathology* (n = *10/56).*

Toxicological survival time (3–12 h)	>	Histological survival time (n = 5) (<1 h)
Toxicological survival time (<1 h)	<	Histological survival time (n = 5) (1–24 h)

Pulmonary histopathology in morphine-associated fatalities also comprises acute and chronic alterations. Acute deaths following intravenous injection of opiates are characterized by the phenomena of congestion/hyperemia, (hemorrhagic) edema and focal hemorrhages (3–5,8,9,11,15–17). The detection of hemosiderin-containing macrophages (siderophages) in the lung tissue is regarded as a correlate for former periods of intra-alveolar bleeding (7,11,13). Further chronic pulmonary changes include the appearance of focal fibrosis, granulomas and giant cells, birefringent material and vascular lesions (7,9,11,12).

Siegel et al. (3–5) established a system of different patterns of pulmonary histology, which attempted to correlate the time interval between the injection and death (see introduction, Table 1). However, this classification appears problematic for several reasons. Only 21 cases with known survival period were investigated. On the one hand the graduation suggests a comparatively high exactness (four stages with temporal references); on the other hand the histological parameters were not rigidly defined. The terms "edema" and "congestion" were not quantified and the meaning of "slight," "small" and "many" was not explained.

Due to their considerable frequency a graduation of these phenomena seems to be difficult. Changes such as edema, congestion or hyperemia, focal hemorrhages, local atelectasis and focal emphysema—often characterized as "narcotic lung" (3–5,16)—are rather nonspecific. They appear in many forms of intoxication and in a number of natural and unnatural fatalities in our own experience. In a recently published study of fatal strangulations we observed similar changes of the pulmonary micromorphology (18). Obviously, for the lung only limited possibilities exist in reaction to a damaging event (in the sense of a common final course, relation to shock lung). This is confirmed by the investigations of Ferrer et al. (19,20). They presented a study on the lung alterations in 66 cases of violent death. The pulmonary lesions were classified into four groups: inflammatory alveolar lesions with and without a diffuse interstitial involvement, hemorrhagic edematous lesions and nonspecific chronic lesions. The complex consisting of hemorrhagic edematous lesions included phenomena of capillary congestion with hemorrhagic extravasation and interstitial and intra-alveolar edema. It was evaluated as the most common lesion which appears very early and can constitute the preliminary stage of any other condition.

In our study the semiquantitative graduation of general microscopic lung alterations brought heterogeneous results; however, several "key parameters" such as edema, alveolar hemorrhages, focal emphysema and intravascular cell accumulations can provide criteria for an evaluation of the survival period. The chosen temporal scheme—with a classification of the survival time in <1 h, 1–24 h and >24 h—had to be relatively broad-based. It was the only possibility to make "certain" use of the anamnestic data available and to obtain significant differences in the expression of the histologic indicators applied. We preferred a broad-based, but more reliable temporal staging rather than a narrow-based, but perhaps incorrect classification.

Under these prerequisites, one could differentiate between cases with more and with less than one hour survival periods following morphine injection: the subgroup with short courses of intoxication (<1 h) was mostly characterized by slight or moderate alveolar edema, severe hemorrhages and marked acute emphysema, while a rather extensive edema, slight hemorrhages and missing or slight

emphysema were striking in the group with intermediate survival times (1–24 h). Intravascular cell accumulations—as a shock equivalent—served as a marker of "longer" (>1 h) courses of intoxication. Only the combination of the examined histological parameters was useful for a temporal assessment.

The time-dependent occurrence of the various phenomena can be explained by the results of shock research (21,22). Acute pulmonary hyperemia or congestion, respectively, belong to the first phenomena of shock and are present within seconds. Subsequently, interstitial and intra-alveolar edema develop. A relationship between shock lung and narcotic lung has been described (16). The exact pathogenesis of lung edema in heroin intoxication is not clear (11). Various factors such as hypersensitivity reactions, hypoxia from respiratory suppression and direct toxic effects of opiates, both with subsequent increase of pulmonary capillary permeability, may play an important part (16,17,22).

The combination of hyperemia, edema and hemorrhages, in addition, is responsible for the very high lung weights in morphine-involved deaths (average of 1700 g in cases with survival times up to 24 h). In this respect there is concordance with data in the literature, where lung weights between 1100 and 2000 g are mentioned (3,15). The higher incidence of marked alveolar hemorrhages in the group with shorter survival periods seems to be paradoxical, but could be related to the special effects of this phenomenon limiting longer survival. The finding of marked focal emphysema in cases with short survival may be explained by disturbances of respiratory regulation with deep terminal breaths; it also may be caused by resuscitation being more frequent in addicts with short survival (positively correlating artefact, thus not leading to an exclusion of such cases). In longer courses of intoxication increasing edema probably overlays emphysema.

We have already discussed the important phenomenon of intravascular cell accumulations and its meaning as a shock equivalent in the connection with fatal strangulations (18). It is a marker of protracted agonal courses. Presumably a "sticking" of granulocytes in pulmonary vessels occurs early during shock, but it takes approximately one hour for a distinct histologic manifestation of this alteration. Therefore, it probably can be used for a differentiation between short and longer courses of intoxication with a limiting value of approximately one hour.

In contrast to Siegel et al. (3) we did not observe hyaline membranes in cases with survival periods <24 h. They appeared only once in a case with pneumonia and a survival of several days in coma. In addition, the phenomenon of bronchopneumonia characterized delayed deaths after >24 h, but could not be used as a relevant parameter of survival period, because it also was found in the group with intermediate survival times.

The study showed that pulmonary histopathology can complete toxicological data on kinetics in brief periods of intoxication. It makes a further differentiation possible within the interval from 0–3 h (model of Spiehler). Multiple heroin or morphine injections, one shortly after another, may lead to misinterpretations of toxicological findings (percent unconjugated morphine in blood decreases, simulation of a longer survival period). The observed differences in the evaluation of survival time between toxicology and pulmonary histopathology (n = 10/56) might be explained as follows: the constellation 1 (toxicology > histology) could be caused by an "overhang" of conjugated morphine from an earlier consumption of opiates. As to constellation 2 (toxicology < histology) it must be mentioned, that the toxicological 1-hour-interval was based on a provisional limiting value of >100 μg/kg 6-MAM in brain tissue. Moreover, a protracted state of shock induced by multiple heroin injections within a short interval can produce a corresponding pattern of lung histology and simulate a longer interval.

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