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The pathology of the adrenal glands in Sudden Infant Death Syndrome (SIDS)*

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Summary. The sudden infant death syndrome (SIDS) is at present based on unknown pathogenetical mechanisms but in industrial nations is the most common cause of death in infancy after the perinatal period. Studies of a large number of adrenal glands in this syndrome have not been reported. Therefore, we evaluated 146 SIDS cases (85 males, 61 females, aged 14–465 days) and 24 control cases (17 males, 7 females, aged 18–623 days) by light microscopy, morphometry and immunocytochemistry (anti-chromogranin A and anti-S100 protein). Our data revealed a normal maturation of the adrenal glands in SIDS cases. Necroses, extensive hemorrhages or inflammation were not found. A focal lipid depletion of the zona fasciculata was seen in 92% of the adrenal glands of the SIDS and control cases. We found a siderosis (in 33% of the SIDS cases and 4% of the control cases) and calcium deposits (13% and 12% respectively) due to hyperemic involution of the fetal zone. The medulla, including the sustentacular cells (S 100 protein-positive cells) and chromaffin cells (chromogranin A-positive cells) was unchanged. Our results indicate that the few morphological alterations of the adrenal glands in SIDS cases are the effect of the underlying disease and not the cause of the sudden death.

Key words: Sudden Infant Death Syndrome – Adrenal glands – Adrenal medulla – Adrenal cortex – Involution zone

Zusammenfassung. Der plötzliche Kindstod (SIDS), dem noch unbekannte pathogenetische Ursachen zugrunde liegen, ist die häufigste Todesursache im 1. Lebensjahr nach der Neugeborenenperiode in den entwickelten Ländern. Beiträge über die morphologischen Veränderungen an einem großen Kollektiv von Nebennieren beim plötzlichen Kindstod sind uns aus der Literatur nicht bekannt. Daher haben wir ein Kollektiv von 146 Fällen (85 männlichen und 61 weiblichen Geschlechts im Alter zwischen 14 und 465 Tagen) und eine Kontrollgruppe von 24 Fällen (17 männlichen und 7 weiblichen Geschlechts im Alter zwischen 18 und 623 Tagen) lichtmikroskopisch, morphometrisch und immuncytochemisch (anti-Chromogranin A und ant-S100 Protein) untersucht. Wir fanden eine regelrechte Reifung in den Nebennieren von den SIDS Fällen. Nekrosen, extensive Einblutungen oder entzündliche Infiltrate bestanden nicht. In 92% der SIDS Fälle und auch der Kontrollgruppe war eine fokale Lipidentspeicherung der Zona fasciculata zu beobachten. Eine Siderose zeigte sich in 33% der SIDS Fälle und in 4% der Kontrollfälle und Verkalkungen in 13 bzw. 12% der Fälle, die wir als Zeichen der hyperämischen Involution der fetalen Zone deuteten. Die chromaffinen Zellen (Chromogranin A-positive Zellen) und die Satellitenzellen (S 100 Protein-positive Zellen) des Nebennierenmarks erwiesen sich als regelrecht. Wir schlußfolgern aus unseren Ergebnissen, daß die wenigen morphologischen Veränderungen in den Nebennieren von SIDS-Fällen Folge der zugrunde liegenden Erkrankung und nicht die Ursache des plötzlichen Todes sind.

Schlüsselwörter: Plötzlicher Kindstod – Nebennieren – Nebennierenmark – Nebennierenrinde – Involutionszone

Introduction

The sudden infant death syndrome (SIDS), defined as "the death of an infant or young child, which is unexpected by history and in whom a thorough necropsy examination fails to reveal a cause of death" [2, 11] is the most common cause of death in infancy outside the perinatal period.

The estimated incidence of SIDS in industrial nations is 1–3 per 1000 live births [3, 9, 11, 16, 19]. In the Federal Republic of Germany about 1000–1500 and in Great Britain around 2000 children die from SIDS annually [11].

All research in this field, whether epidemiological, microbiological or chemical, depends on the accuracy of the autopsy diagnosis for its validity. It is the anatomical pathologist who takes the responsibility to diagnose death

* Dedicated to Prof. Dr. J. Kracht on the occasion of his 70th birthday

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due to SIDS if the cause of death cannot be explained adequately [19, 20].

Since Garrow and Werne [8] published some of the earliest scientific investigations on this subject the concept of pathological findings and pathophysiological reactions in SIDS have been constantly modified with the increasing number of cases investigated [1]. Today most scientists recognize the multifactorial nature of SIDS. Different studies have demonstrated morphological differencies between healthy infants and SIDS cases in post-mortem examinations [14, 19, 20]. Among others, alterations of the pulmonary arteries [20], right ventricle [20], liver [20], glomic tissue of the carotid body and chromaffin tissue in the adrenal medulla [14] and an abnormal gliosis in the brainstem [14] have been described. These results have been interpreted as alterations due to chronic hypoxemia. The most common morphological findings in SIDS are localized in the upper and lower respiratory tract, in the nervous system, thymus and in the lymphatic tissue [14, 19, 20].

In the last ten years many groups of forensic and pediatric pathologists have developed different autopsy protocols to provide a basic standard for an adequate post-mortem examination [10, 19, 20, 21]. Despite the published protocols, which include the evaluation of the adrenal glands [10, 19, 20, 21], there are only a few reports in the literature on morphological changes in the adrenal glands in SIDS [1, 14]. However, it is well known that an adrenal insufficiency can remain clinically undetected until an adrenal decompensation takes place and the patient dies suddenly.

In 28 cases of SIDS Naeye [14] found an enlarged mass of chromaffin cells in the adrenal medulla as an indication of chronic hypoxemia. Althoff [1] emphasized that further research would be necessary to clarify the role of the adrenosmypathetic system in SIDS.

The aim of our study was to investigate the features of the adrenal glands in a large number of SIDS cases using histological, morphometrical and immunocytochemical methods, and was part of a larger multicenter study.

Material and methods

I) The study group

The study group included 146 cases of SIDS, aged between 14 and 465 days (mean age 156.2 days). The sex distribution was 85 (58%) males and 61 (42%) females (Table 1). The control group included 24 cases, aged between 18 and 623 days (mean age 209.3 days). The sex distribution of the control group was 17 (71%) males and 7 (29%) females (Table 2). The causes of death of the control group are shown in Table 3.

The adrenal glands for this study were obtained from the Institutes of legal Medicine at the Universities of Münster and Essen (Germany).

II) Histology

The adrenal glands were fixed in formalin and embedded in paraffin. The slides were stained with HE, PAS, prussian blue, von Kossa's method for calcium deposits and Gomori's silver staining method for reticulin. The morphometric data of the cortex were obtained with an eyepiece micrometer scale (Leitz, Wetzlar).

Table 1. Cases of death due to SIDS

Age in days	Males	Females	Total
1– 90	31	18	49
91–180	27	21	48
181–270	18	9	27
271–360	9	8	17
>361	8	5	5
Total	85	61	146

Table 2. Control cases

Age in days	Males	Females	Total
1– 90	4	0	4
91–180	4	2	6
181–270	6	2	8
271–360	0	2	2
>361	3	1	4
Total	17	7	24

Table 3. Causes of death in the control group

Cause of death	Number of cases	Percentage
Pneumonia	7	29.1
Drown's syndrome	4	16.6
Suffocation	2	8.3
Carditis	2	8.3
Sepsis	2	8.3
Strangulation	1	4.2
Tracheobronchitis	1	4.2
Hyperthermia	1	4.2
Ileus	1	4.2
Vitium cordis	1	4.2
RDS III	1	4.2
Reflectory death	1	4.2
Total	24	100.0

III) Immunohistochemistry

Adrenal glands containing part of the medullary tissue (100 in the SIDS group and 20 in the control group) were immunostained for chromogranin A and S100 protein by the avidin-biotin-peroxidase complex technique. In those cases where no medullary portion could be found, it would seem that this is a problem of sampling and not a sign that the adrenal medulla was absent.

The sections were initially incubated with a 1:50 dilution of normal goat serum (30 min, room temperature) and then with rabbit-anti-S100 protein (polyclonal, Dako, Hamburg, FRG, 1:400) or mouse-anti-chromogranin A (monoclonal, Boehringer, Mannheim, FRG, 1:100) antibodies (30 min, at room temperature). The sections were incubated with biotinylated anti-rabbit or anti-mouse antibodies and finally with preformed avidin-biotin-complex (ABC). The reaction product was visualized with diaminobenzidine tetrahydrochloride as chromogen. All sections were counterstained with Mayer's hematoxylin.

IV) Quantification and classification

Adrenal cortex. Permanent cortex: The morphology and stage of development of the permanent cortex were graded as follows: the zona glomerulosa as: not present/in development/well developed; the zona fasciculata in relationship to the degree of lipid depletion as: no lipid depletion/focal lipid depletion/generalized lipid depletion.

Fetal cortex: The thickness of the fetal cortex was measured morphometrically in 6 different areas of the adrenal glands and was expressed as the ratio: fetal cortex/total adrenal cortex (%). Hyperemia of this zone was classified as: none/mild/severe/hemorrhages. Siderosis and calcium deposits were investigated by prussian blue and von Kossa staining and classified as: absent/mild/partial/generalized.

Adrenal medulla. The adrenal medulla was evaluated for its content of chromaffin and sustentacular cells (chromogranin A-positive and S100 protein-positive cells, respectively). The presence and degree of development of a medullary capsule were verified by staining the argyrophil fibers by Gomori's technique.

Results

In our collective of 146 SIDS cases the mean age was 156.2 days (14 to 465 days), and 98% died before the end of the first year of life and 67% before the end of the sixth month (Table 1).

Table 4. Ratio: fetal cortex/total adrenal cortex (%) in SIDS cases

Age in days	0–15%	16–30%	>31%	Total
1– 90	3	21	25	49
91–180	12	31	5	48
181–270	14	13		27
271–360	10	7		17
>361	4	1		5
Total	43	73	30	146

The zona glomerulosa was identified as a narrow band of small clear cells beneath the capsule, which was not distinctly separated from the zona fasciculata in most (71%) SIDS cases.

The zona fasciculata, which was well developed in all cases, consisted of columns of spongiocytic cells with typical ovoid nuclei and a lipid-rich clear cytoplasm. In the majority of cases (92%) small clusters of cells were found with more eosinophilic, smaller cytoplasm and ovoid dark nuclei as a typical sign of lipid depletion.

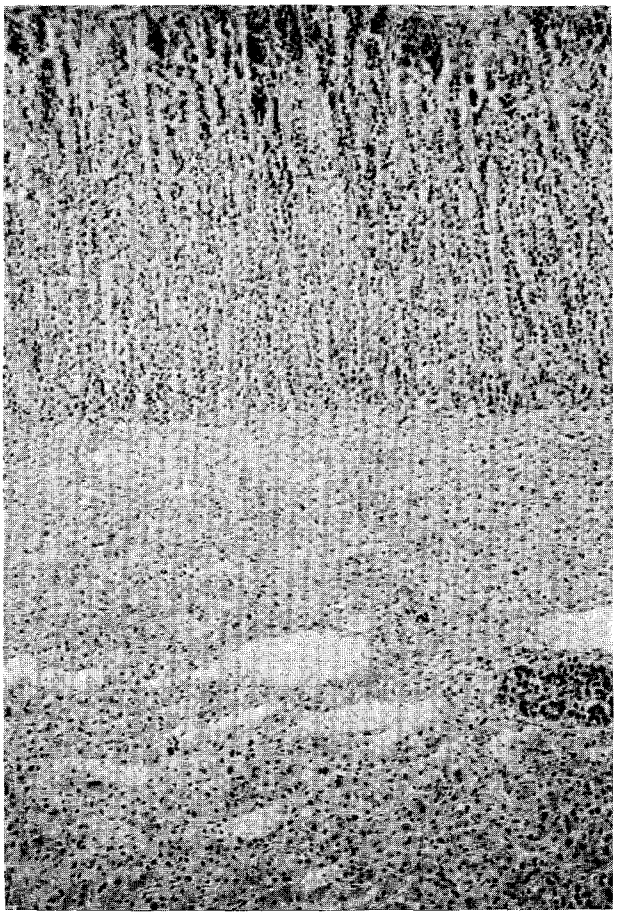

Fig. 1. Adrenal gland from a 121-day-old boy: regular permanent cortex, involution zone *(below)* with involution alterations. Hematoxylin-Eosin (× 110)

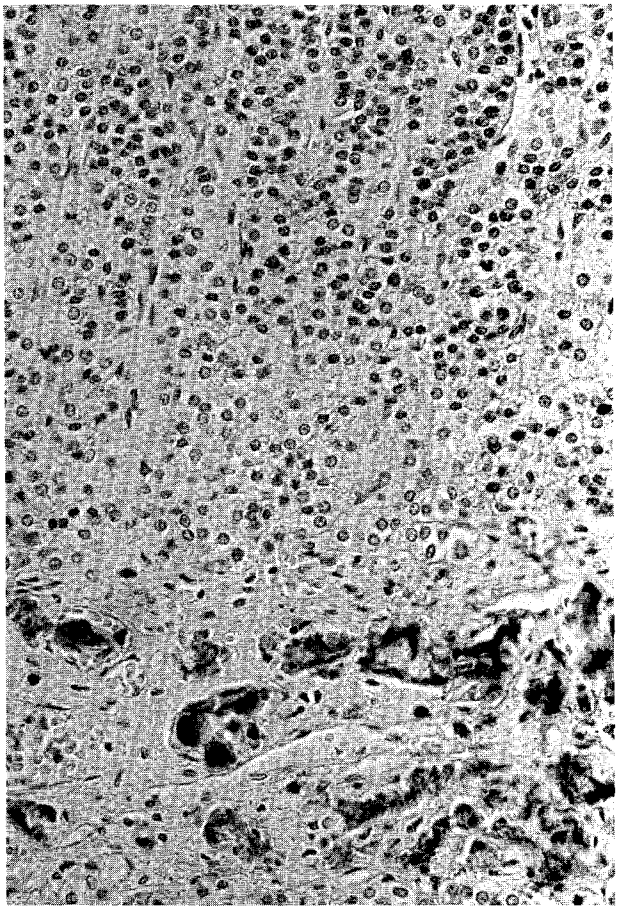

Fig. 2. Adrenal gland from a 320-day-old girl: regular permanent cortex, strong siderosis in the involution zone *(below)*. Prussian blue (× 270)

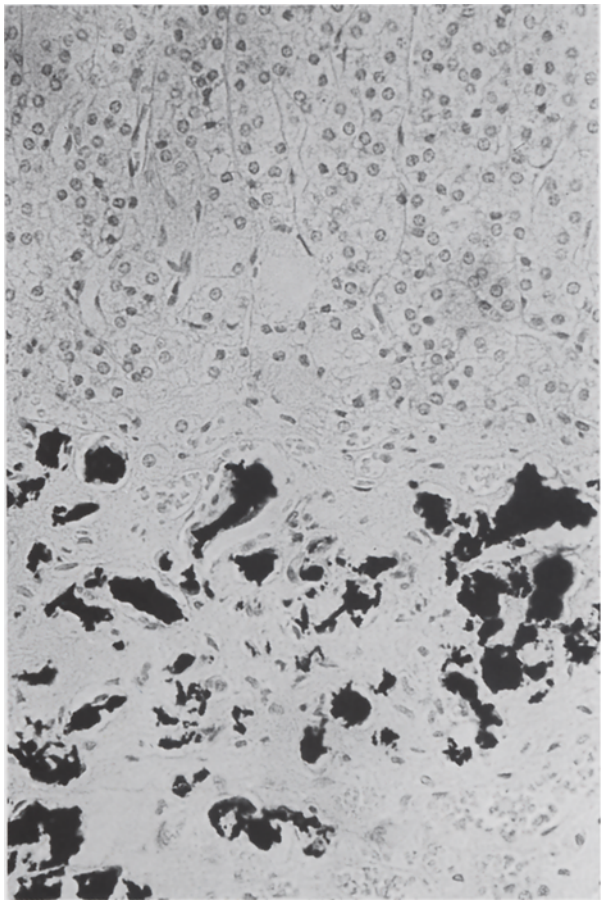

Fig. 3. Adrenal gland from a 320-day-old girl: regular permanent cortex, calcium deposits in the involution zone. Von Kossa's reaction (× 270)

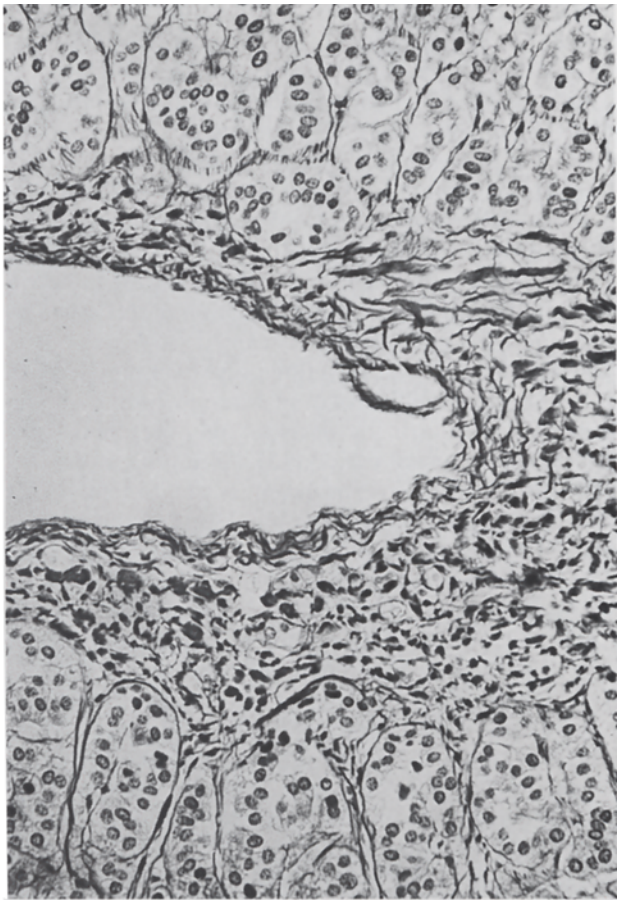

Fig. 4. Adrenal gland from a 40-day-old girl: involution zone and dense network of reticulin fibres as a medullary capsule around a medullary vein. Gomori's silver method (× 270)

The sinusoids of the cortex were regulary shaped without thrombosis, hemorrhages or any other alterations. Signs of extensive fibrosis or necrosis were not found in the permanent cortex.

The fetal zone consisted of lipid-rich cells among a well developed capillary network (Fig. 1).

A progressive reduction in size was observed during the postnatal period due to the postnatal degeneration of this zone (Table 4). A mild hyperemia of this zone was seen in 42% and a severe hyperemia in 58% of the SIDS cases and in 63 and 37% of the control cases respectively.

A siderosis of the fetal cortex appeared in 37% of the SIDS cases and in 4% of the control cases (Fig. 2) and a calcification in 12 and 13% respectively (Fig. 3).

The adrenal medulla exhibited a strongly positive, homogeneous immunostaining for chromogranin A in all cases. Sustentacular cells, positive for S100 protein, were randomly scattered among chromaffin cells but were clearly less numerous.

The medullary capsule initially was seen as a long broad band of reticular connective tissue, which gradually became narrower and finally, by the end of the first year of life, sharply delineated the medulla and the permanent cortex (Fig. 4).

Discussion

The majority of cases occurred in the first year of life (98%) with a maximum between the second and fourth month (Table 1). A comparison of the frequency of SIDS before the end of the sixth month of life in our group with that reported in the literature shows similar results [1, 12].

We observed a preponderance of the male sex in our group (Table 1). Similar results have been obtained from other large epidemiological investigations of SIDS [1, 12]. Some authors also observed a preponderance of the female sex in their collectives but these differences could not be explained [1].

Despite many efforts during the last decade to try to define epidemiological features which could be relevant to SIDS and to identify infants at risk, there have been only very few morphological studies of the adrenal glands in SIDS [1, 14].

However, the development and maturation of the adrenal cortex in healthy children dying from accidental causes has been adequately studied [5, 6, 7].

In the adrenal cortex of infants there is no sharp demarcation between the zona glomerulosa and fasciculata and both layers can only be distinguished by their cellular features [15]. The zona glomerulosa becomes developed

and separated from the zona fasciculata only after the end of the second year of life. This is in accordance with our observations.

The focal lipid depletion of the zona fasciculata, present in 92% of SIDS cases, was interpreted as a sign of activity with high hormone release, as defined by Dhom [5, 6]. In 1956 Symington et al. [17] reported a focal lipid depletion in 90–95% of unselected post-mortem examinations and a massive lipid depletion of the adrenal cortex of children dying from severe infections or burns. In accordance with this we found a lipid depletion of the zona fasciculata in 92% of our control cases.

The fetal cortex or involution zone occupies 75% of the adrenocortical thickness in mature newborns. The involution with hemorrhagic necrosis may be rapidly progressive in the neonatal period [15]. At the end of the first month of life the ratio of permanent cortex to fetal cortex decreases to 1:1 [4, 5, 13]. The involution of this zone is completed by the end of the first year [5, 6].

The size of the fetal cortex in our study exhibits an inverse relationship to the postnatal age (Table 4). The siderosis and calcium deposits found in this zone can be correlated to the hyperemic involution process.

The collapse of the stroma, which accompanies the involution of the fetal cortex, results in the formation of a medullary capsule. The long broad band of connective tissue seen in the first months, gradually narrows and sharply delineates the medulla and permanent cortex by the end of the first year of life [5, 6, 15]. In accordance with this phenomenon we found a good correlation between the age and the degree of development of the medullary capsule in our collective.

Despite extensive studies of the adrenal cortex in childhood, systematic studies on the development of the adrenal medulla have not been performed [1], but the degree of maturation of the medullar cells seems to be largely incomplete during the first year of life. The maturation of this zone is said to be complete only after the end of the second year [5, 6, 15].

By the immunohistochemical studies we could not find any abnormalities in the medulla. The chromaffin cells were seen as an homogeneous, strongly chromogranin A-positive mass of cells around the central vein. The sustentacular cells, randomly scattered among the chromaffin cells, were typically stained positive for the S100 protein [18].

Furthermore, we did not observe any inflammation, tumors, extensive necrosis, hemorrhages, hypoplasia, or other alterations in the adrenal glands from the 146 SIDS cases.

Conclusions

The process of maturation in the adrenal medulla as well as in the adrenal cortex of SIDS cases appears to be normal. The hyperemia, siderosis and calcium deposits in the involution zone can be interpreted as signs of the involution process.

No morphological alterations were observed in the adrenals of our 146 SIDS cases which could explain the pathogenesis or the cause of death in this syndrome. The few morphological changes of the adrenals must be interpreted as an effect of the unknown underlying disease and not as a cause of the sudden death and as they were also present in the control cases.

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