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Peliosis of the Female Adrenal Cortex of the Aging Rat*

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Summary. Foci of apparent peliosis are regularly observed in the mid-zone of the adrenal cortex in female rats older than 600 days. The changes present range from ectasia of the sinusoids to extensive cystic change of the whole organ.

This lesion occurs almost exclusively in female animals and was seen in only one of 50 male animals older than 600 days examined. Experimental stimulation or inhibition did not influence adrenal peliosis. Electron microscopically, there was marked pericapillary edema with collapse of the capillaries, and erythrocytes and thrombocytes were seen infiltrating the edema. Fibrin accumulated in the larger foci. Degenerative alterations were not observed either in the epithelial cells of the cortex or in mesenchymal cells. The pathogenesis is unknown, but the possible role of constant estrus in aging female rats will be discussed.

Key words: Peliosis – Female adrenal cortex – Age

Introduction

Peliosis hepatis means a focal cavern-like dilatation of the liver sinusoids forming lakes of blood, which sometimes involves a whole lobule. Recently, this lesion had been observed more frequently. Often long-term treatment with anabolic steroids precedes the development of this lesion (Altmann and Klinge 1972; Klinge 1979; Nadell and Kosek 1977). Peliosis of the liver – and also of the adrenals – has been described in mice under experimental conditions, in which a granulosa cell tumor of the ovary had been implanted (Furth 1946). Wayss et al. (1979) describe a pronounced peliosis hepatis in praomys (mas-

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** Dedicated to Professor Hans-Werner Altmann on the occasion of his 65th birthday

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tomys) natalensis following the short-term administration of dimethylnitrosamine.

For the first time in 1975, we called the attention to the fact that pronounced alterations closely resembling peliosis hepatis can be observed frequently in the adrenal cortex of aging female rats (Seebach et al. 1975). Until now, only a few morphologic studies of the adrenal cortex of the aging rat have been published (Tobin and Whitehead 1942; Jayne 1953; Reichel 1968; Szabo et al. 1970; Stöcker and Schmid 1973; Nickerson et al. 1979) and peliosis has never been described. For this reason, in the present study we have analysed the frequency, sex distribution and morphology of this age-related change in Chbb: TOM (SPF) rats, these being the variety at our disposal.

Material and Methods

200 (100 male and 100 female) albino-rats of the breed Chbb:TOM (SPF) were kept in fully air-conditioned rooms (room temperature of $22 \pm 1°$ C and constant light and darkness rhythm) under specific pathogen-free conditions. The animals were fed with an Altromin-R-fortified diet, with food and water ad lib. 50 female and 50 male animals were sacrificed at an age of 90 days and the same number of animals at an age of 600–639 days. Before sacrifice, 10 animals in each of the four groups were submitted to the following:

1. ACTH-depot (Schering) in gelatin 20 i.u./kg body weight were given subcutaneously daily for 11 days.
2. Gelatin 0.5 ml/kg body weight were given subcutaneously daily for 11 days to a control group.
3. Corticosterone 20 mg/kg body weight were given intraperitoneally: in some animals daily for 2 days and in others daily for 7 days.
4. Animals were subjected to immobilisation stress for 5 h each day for 11 days.

The animals were sacrificed immediately after completion of these procedures. Blood plasma was taken for aldosterone and corticosterone determinations (Laboratory of Prof. P. Vecsei, Institute of Pharmacology, Heidelberg). The organs were removed and weighed immediately after death. The right adrenal was fixed in Bouin's solution and embedded in paraffin wax. The left adrenal was fixed in glutaraldehyde and embedded in Araldite for electron microscopy. The adrenal weights were evaluated statistically.

Results

A. Light Microscopic Findings

Of the animals sacrificed at the age of 90 days, one of the 50 females, but none of the 50 males showed changes of adrenal peliosis. Of the animals aged 600–639 days, peliosis was seen in only one of the 50 males, but was present in all the 50 females to a varying degree. In the one older male animal, peliosis was associated with a circumscribed necrosis of the adrenal cortex. The various pretreatment regimes – ACTH, corticosterone, stress – did not influence the extent or character of the peliosis. A report will be published elsewhere on the morphology and function of adrenal cortical cells after stimulation and inhibition at different ages.

Microscopically, peliosis of the adrenal cortex in females aged more than 600 days could be classified into 4 grades according to its severity (Fig. 1):

Grade 1. The peliosis displayed localized ectasias of sinusoids in the mid-fasciculata. Focally, the sinusoids formed a network, the greater part of which was

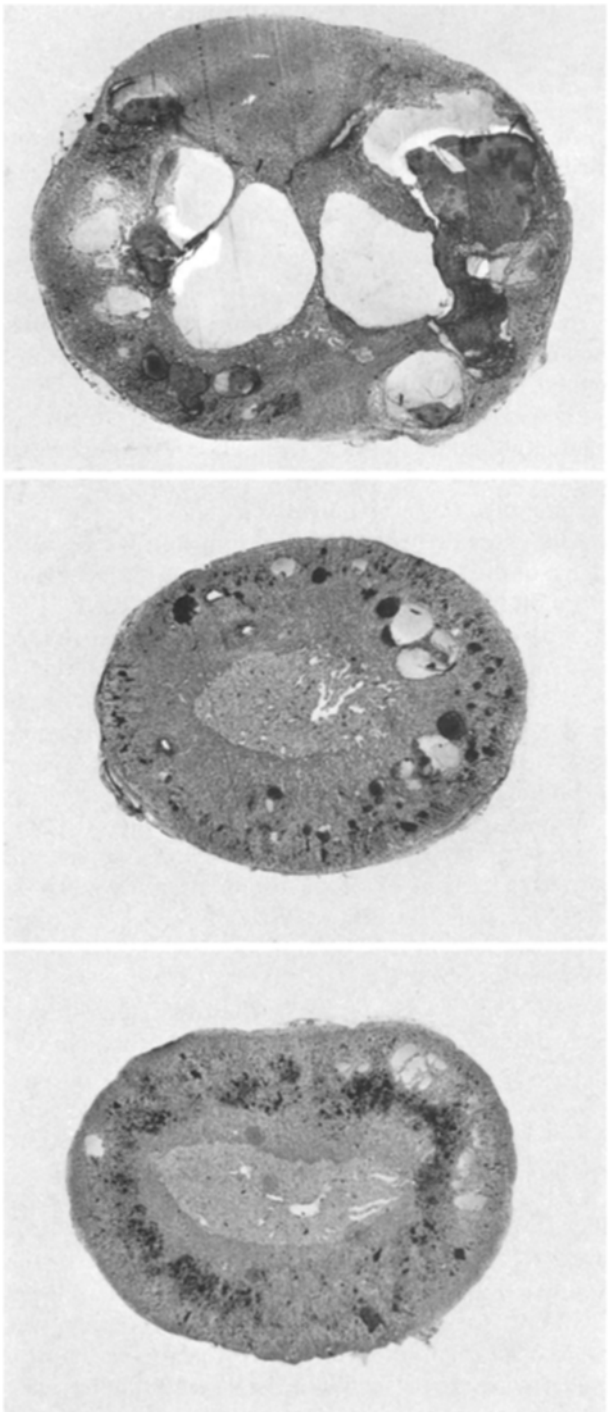

Fig. 1. Peliosis of the adrenal cortex. Different grades of severity. 600-day-old female rats. Goldner. Magnification about $\times 12$

filled with blood. The more dilated sinusoids appeared rounded. There were not significant changes in the sinus-lining cells or the adjacent parenchymatous cells (8 animals).

Grade 2. The peliosis displayed more pronounced vascular ectasia, with more frequent rounded cavities filled with plasma and erythrocytes (14 animals).

Grade 3. Besides a network of widened sinusoids, the peliosis showed cystic spaces to form a system of cavities (21 animals).

Grade 4. Almost the whole of the adrenal was transformed into large, blood-filled cysts, producing an enlargement of whole organ. In addition, smaller cystic cavities could be seen (7 animals).

The changes were always most pronounced in the mid-cortex. A narrow strip of the outermost fasciculata and glomerulosa was present on the superficial aspect of even the largest cystic cavities, and the component cells showed no evidence of compression. The medulla was never involved, and the medullary vessels remained unchanged. The vascular walls and the sinus-lining cells were intact, as long as the basic pattern of the sinusoids was preserved. With increasing dilatation, the spaces were more often lined by cortical parenchymal cells. Frequently, there was separation of plasma and erythrocytes in the cystically dilated cavities, and a fine network of fibrin could be seen in the blood. The ectatic sinusoids formed irregular lakes into which plates and trabeculae of parenchymal cells projected as peninsulas and narrow spits (Fig. 2). The adjacent parenchymatous cells showed an intact, regular nuclear pattern. The cytoplasm often revealed large fatty vacuoles, a feature regularly observed in the deeper cortical layers of the adrenal cortex of aging animals, even in the absence of peliosis. Often there was a diffuse leakage of blood between the groups of parenchymal cells at the margins of the smaller cavities, but evidence of cytolysis or necrosis of these parenchymal cells was lacking. The larger cystic cavities were often partially bounded by a thin fibrous membrane. Thrombosis, granulation tissue, or hemosiderin deposition was never observed. No tumors of the cortex or medulla were noted in the female animals. However, in the older (600+days) male animals without peliosis, 6 tumors of the medulla and one cortical adenoma developed.

B. Electron Microscopic Findings

The adrenal sinusoidal capillaries were very similar in construction to those of the liver sinusoids described by Ito and Shibasaki (1968). However, the flat endothelial cells did not possess any pores, but only fenestrations closed by a thin membrane. The narrow, partially dispersed basal membrane was not always evident. In only a few cases, monocytes were seen in the sinuses. Thin fibrocytes and macrophages were present beneath the subendothelial cells. They were situated parallel with the endothelial cells, in small recesses between adjacent cortical cells, their ends extending close to the endothelium. The suben-

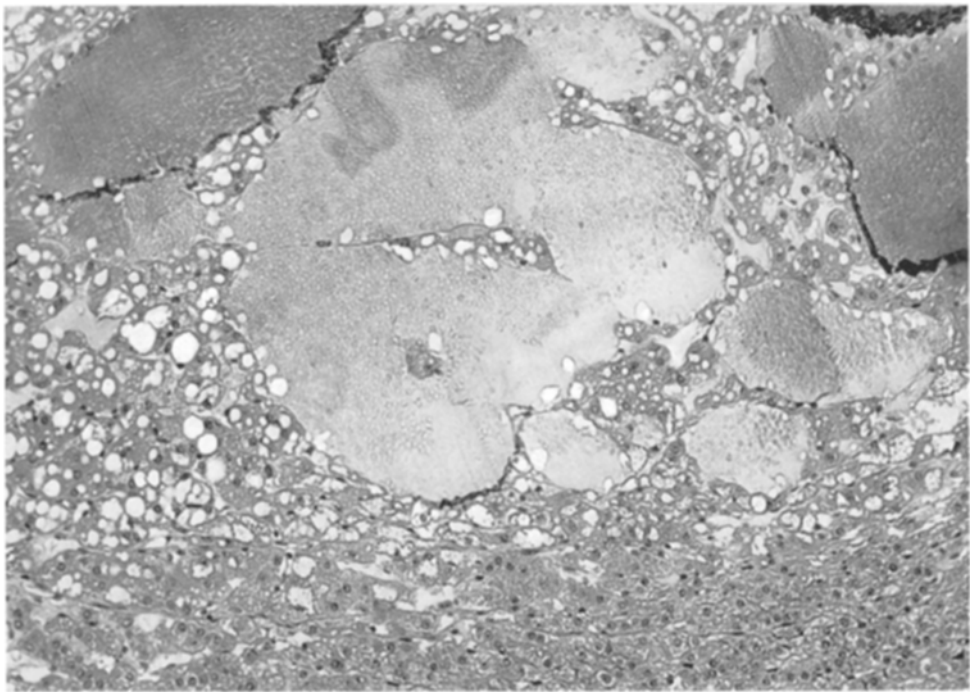

Fig. 2. Peliosis of the adrenal cortex. Polymorphic lakes of blood plasma. Cortical epithelial cells, which are rich in lipids, in the deep cortical layers. Goldner. Magnification × 150

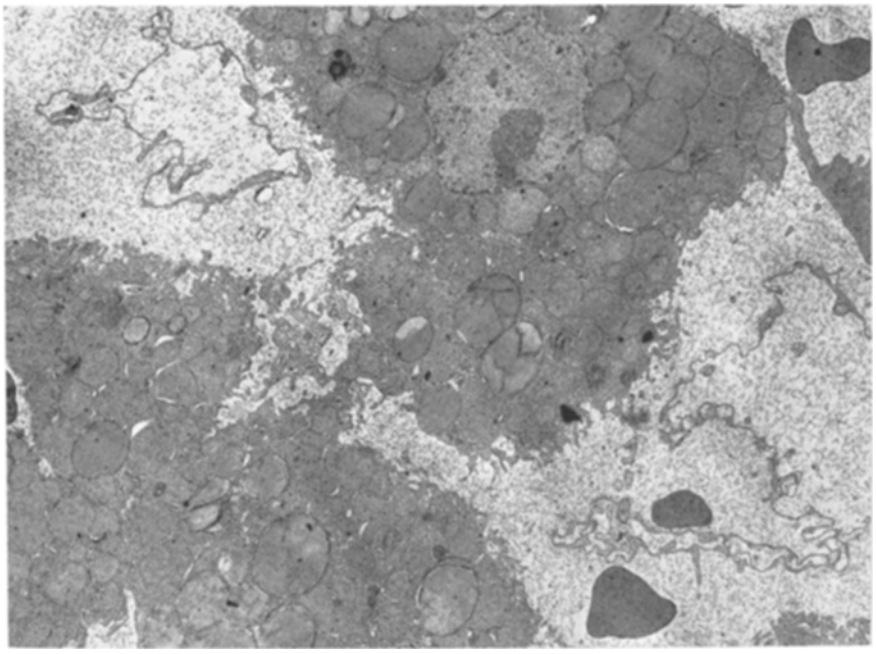

Fig. 3. Perisinusoidal edema with collapsed capillaries. Intact fasciculata cells. Magnification × 3,600

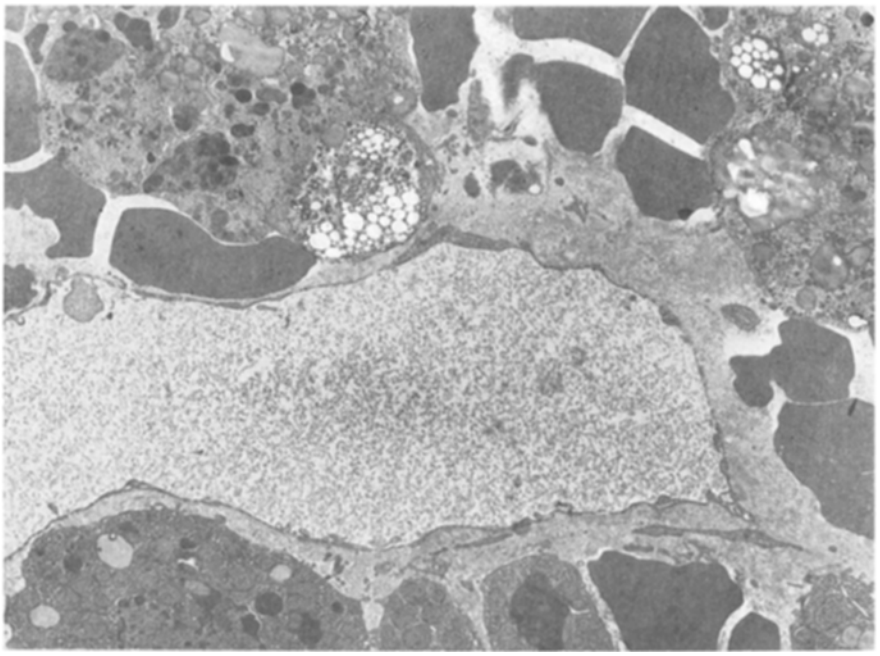

Fig. 4. Broadened pericapillary space with erythrocytes and densification of ground substance. At the upper margin of the picture are macrophages loaded with lipids. Magnification ×4,500

dothelial macrophages stored lipids. In old animals, these cells were increased in number and frequently they were loaded with, and distended by residual bodies formed from phagocytosed cortical cells.

There were no unequivocal nuclear or cytoplasmatic changes in the endothelial cells, fibrocytes or macrophages in the foci of peliosis. The most striking alteration was a pronounced pericapillary edema which produced considerable broadening of the periendothelial zone as well as loosening of the cortical cell complexes (Fig. 3). At first, the capillaries were widened or dilated. With increasing edema, the capillaries showed a markedly folded circumference and were occasionally completely collapsed. Pericapillary, cyst-like cavities developed, and became filled with edema fluid, which was poor in protein. Focally in the extracapillary space, one could make out accumulated electron-dense material. The cavities were demarcated by inconspicuous cortical cells and in a few places by stretched sinusoids. Communications between the sinusoids and the dilated cavities could not be seen. Sometimes gaps were present in the endothelial lining and in the basal membrane, but these could be due to artefacts caused during preparation. Erythrocytes and sometimes thrombocytes were present in the interstitium, but their presence bore no obvious relation to the extent of sinusoidal dilatation or pericapillary edema (Figs. 4, 5). Focally, fibrin precipitates were also present (Fig. 6). Erythrocytes were seen most often in the subendothelial space. They were also found between the adjacent cortical cells, indicating the presence of passages through which erythrocytes had escaped from the adjacent capillaries. It appeared that in the marginal areas of the

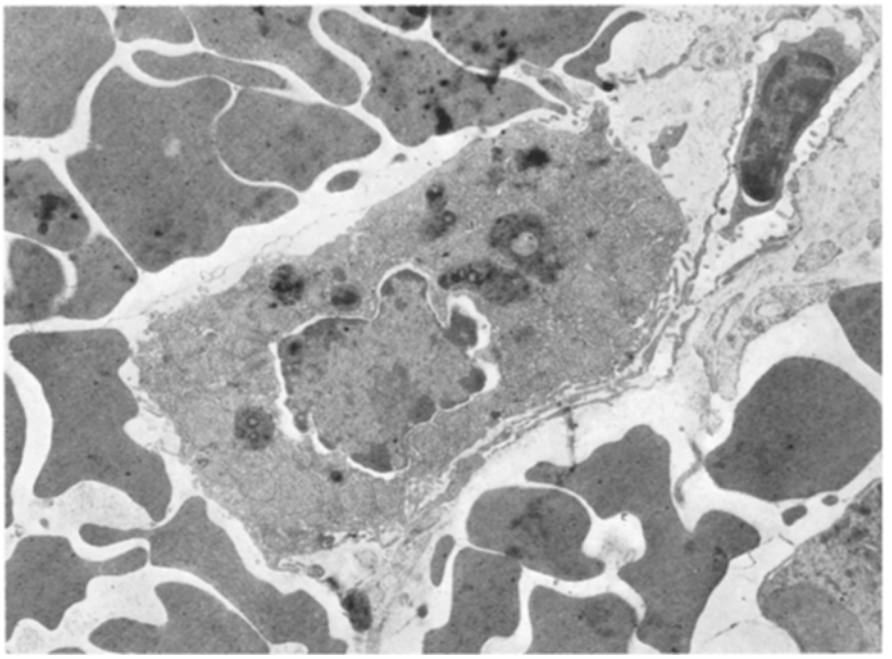

Fig. 5. Well-preserved reticularis cell situated below a completely collapsed capillary. Numerous pericapillary erythrocytes. Magnification × 5,040

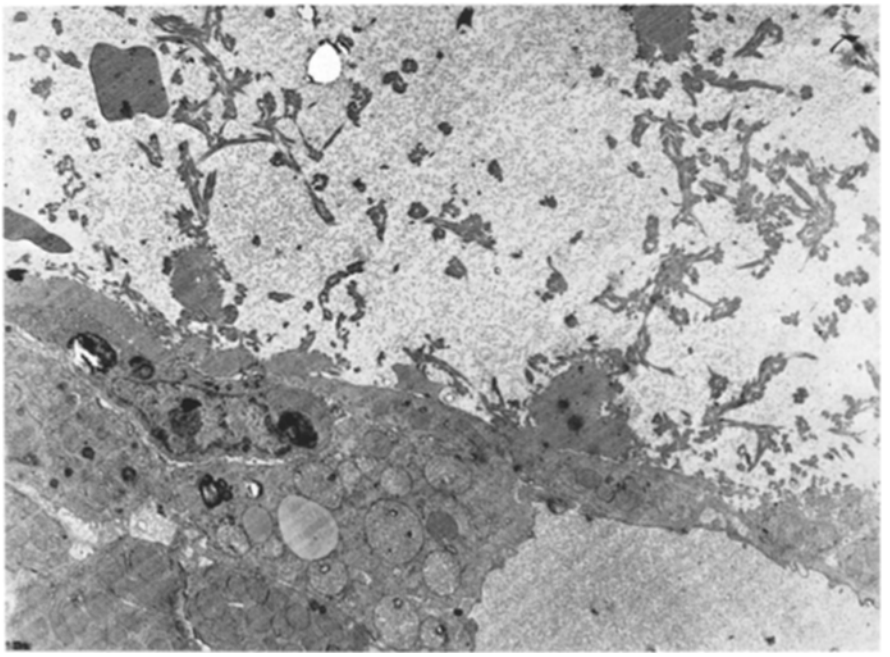

Fig. 6. Margin of a large focus of peliosis with pericapillary fibrin. Cortical cells, which are extended in length, between capillary (*top*) and focus of peliosis. Magnification × 3,780

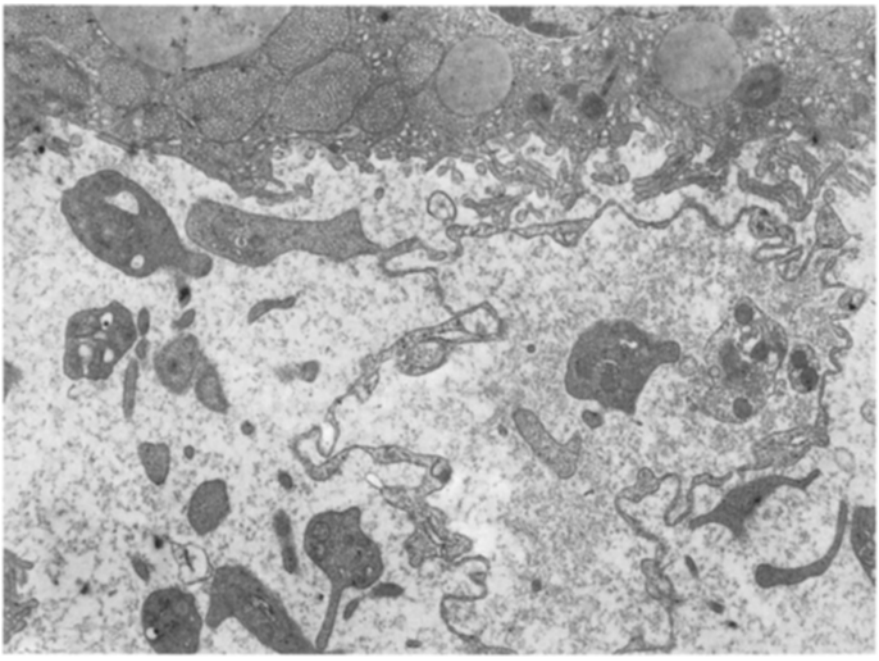

Fig. 7. Pericapillary edema with thrombocytes. Abundant microvilli of an intact cortical cell (*top*) extend into a lake of edema fluid. Magnification ×10,560

hemorrhagic foci, erythrocytes had been pressed into the subendothelial space where pericapillary edema had not been previously present. Only some erythrocytes were phagocytosed by perisinusoidal macrophages.

The majority of cortical cells in the foci of peliosis showed no significant nuclear or cytoplasmic abnormalities (Figs. 5, 7). Very occasional cells showed evidence of intracellular metabolic disturbances, with the development of giant mitochondria, intramitochondrial vacuoles or fatty degeneration. Even those cells apparently completely surrounded by blood lakes contained organelles which lacked any obvious pathological alteration.

The large foci of peliosis contained large amounts of precipitated fibrin. This was partly bound by cortical cells, and partly by a layer of fibrocytes. Locally, the fibrin webs were infiltrated by erythrocytes, arranged apparently in passages, as if the capillary network were still present. In the large massues of fibrin, however, capillaries were absent and only the occasional hyperchromatic remnants of destroyed cells could be found.

C. Adrenal Weights

The mean adrenal weight increased with the degree of peliosis (Table 1). The difference between grade IV and the other grades is significant ($P=0.001$). The sex-specific weight difference in favour of the females was unaltered by age. The 600+-day-old male controls showed a mean adrenal weight of 84 mg

Table 1. Female rats, 600+days old. The mean adrenal weights (mg) associated with the four grades of adrenal peliosis (I–IV) are shown

Grade I	II	III	IV
103.45	125.42	141.78	225.08[a]

[a] $P=0.001$

(53.0–118.3), the 600+-day-old females had a mean weight of 136.92 mg. The large weight range observed in females (between 81.0 and 234.1 mg) was related to the varying degrees of peliosis present.

D. Plasma Corticosterone

The values of plasma corticosterone was nor related to the severity of peliosis present ($\bar{x}=13.3$–20.3 µg/100 ml). In advanced age, the females showed higher plasma corticosterone values than the males. In old females of the control group (gelatin injection) the mean value reached 30 µg/100 ml, whilst in old males it was about 15 µg/100 ml ($P=0.05$; Wilcoxon Test). In the younger controls the same sex difference was present($\bar{x}0=11.3$ µg/100 ml. $\bar{x}♀54.6$ µg/100 ml. $P=0.001$).

Discussion

The morphology of the changes we describe in adrenal glands is evidently very similar to those of the betterknown peliosis hepatis (Jaffé 1923; Senf 1939; Zack 1950; Yanoff and Rawson 1964; Naeim et al. 1973; Nadell and Kosek 1977). Parenchymal necrosis, which in the liver is associated with the parenchymal type of peliosis (Senf 1939; Yanoff and Rawson 1964) was not apparent in the adrenal cortex. Our findings correspond rather with the classic description of peliosis hepatis, as reported by Jaffé (1923). Peliosis in adrenal glands begins with an ectasia of the sinusoids in the mid-cortex. As it increases, however, marked pericapillary edema with broadening of the periendothelial space appears. Electron microscopically, it is very impressive to observe the collapse of capillaries which occurs in the edematous areas. These are limited by parenchymal cells. The same observation had been made in peliosis hepatis by Ross et al. (1972), and Nadell and Kosek (1977). The Disse's spaces expand and the liver epithelial cells demarcate the accumulations of fluid. Pathological changes in the adrenal cortical cells were not observed, and their microvilli appeared very well developed. The escape of thrombocytes, erythrocytes, and fibrin into the pericapillary gaps appears to be a secondary phenomenon. The pericapillary edema could be the result of an increased capillary permeability. Two factors may stimulate the increased accumulation of fluid:

1. the retardation of blood flow in ectatic sinusoids.

2. an increased molarity in the pericapillary space, possibly indicated by basal membrane-like densifications.

No cellular lesions are present in the parenchyma, in the mesenchyme, or in the endothelium. Similar observations were made by Ross et al. (1972) in human peliosis hepatis. Lysosomal storage phenomena are prominent in macrophages, but this is a normal finding in aging animals particularly in the deeper layers of the adrenal cortex.

The pathogenesis of peliosis hepatis is unknown. It has become obvious that the development of this lesion is related to androgenic, anabolic steroid therapy (Altmann and Klinge 1972; Nadell and Kosek 1977) and that estrogens (Knapp and Ruebner 1974), and oral contraceptives may also be responsible. Peliosis can be observed in the neighbourhood of liver cell tumors. In animal experiments changes resembling those of hepatic peliosis have been seen infrequently in the adrenal glands of the spleen of mice following the implantation of a granulosa cell tumor (Furth 1946). The increased estrogen levels produced by the tumors in these animals resulted in a massive increase in blood volume. Wayss et al. (1979) induced peliosis without parenchymal cell changes in mastomys following short-term treatment with the carcinogen dimethylnitrosamine. As some of these animals developed hemangiosarcomas, the authors were inclined to consider peliosis as a benign hemangioendothelioma. Ross et al. (1972) and Nadell and Kosek (1977), on the other hand, considered peliosis hepatis to be a drug-dependent primary lesion of the sinus. It is difficult to equate these observations in human and experimental peliosis hepatis with our findings of spontaneous peliosis of the adrenal cortex occurring on aging female rats, although without doubt, the findings are morphologically identical. When considering the pathogenesis of this adrenal peliosis, two facts have to be pointed out:

1. the lesion occurred only in females, and

2. it is seen regularly only in aging animals.

The sex dimorphism of the adrenal cortex of the rat is well known (Dhom et al. 1971). Sexually mature females have heavier adrenal glands, a larger volume of fasciculata cells with increased smooth endoplasmatic reticulum and a higher mitrochondrial volume (Mäusle 1971). These functionspecific parameters increase during estrus, in comparison with animals in diestrus (Mäusle and Scherrer 1974). Castration of females on the 4th day of life leads to a reduction of the functionspecific morphometric parameters (volume of the individual cell, volume of SER, total volume of mitochondria) below the values of the diestrus animals (Mäusle and Scherrer 1974). The function of the adrenal gland is influenced by the sex hormones at different levels. Female animals show higher values of plasma corticosterone and a shorter biological half-life of cortical steroids (Kitay 1961; 1963; Kitay et al. 1971). The metabolism of corticosterone in the liver is different with regard to age and sex specificity. The sex difference develops during puberty and can be prevented by gonadectomy (Schriefers et al. 1972). Females have an essentially higher delta-4-5-alphahydrogenase activity in the liver (Schriefers et al. 1972). In both males and females, the 5-alpha-reductase of the adrenal gland increases after castration, thereby causing a

rise in the conversion of corticosterone to 5-alpha-dihydrocorticosterone and 3-beta, 5-alpha-tetrahydrocorticosterone (Colby and Kitay 1972; Colby 1978). Finally, estrogen receptors have been identified in the adrenal cortex of rats (Cutler et al. 1978).

There is no doubt that sex hormones are responsible for the differences in function and structure of the cells of the adrenal cortex in male and female rats, although many of the details of the mechanism by which sex hormones influence the adrenal cortex have still to be clarified. Our study group is investigating, whether or not these relationships change with age (Dhom et al, 1978; 1980). With increasing cellular volume the pattern of the male fasciculata cells resembles more closely the female pattern. The female fasciculata cells remain essentially unaltered with increasing age. In both sexes, the cells of the zona reticularis develop a significant increase of the volume. No reduction occurs in the resting plasma corticosterone level. Ovarian function in the aging rat is subject to a considerable change. The regular cycle is lost, and a permanent estrus is often observed (Huang and Meites 1975; Aschheim 1976). In both sexes the neuro-endocrine control of the pituitary – gonad axis shows significant alterations (Miller et al. 1979; Pirke et al. 1979). In the male animal it causes a decrease in plasma testosterone (Pirke et al. 1979) and consequently a partial feminization of the fasciculata cells (Dhom et al. 1978; 1980), but in the female it leads to excessive estrogen exposure of the adrenal cortex. Taking into consideration the general effects of estrogens in the circulation, particularly their ability to cause hyperaemia and to increase capillary permeability for water and electrolytes in connective tissue (Diczfalusy and Lauritzen 1961), the induction of peliosis under conditions of permanent estrus is readily understandable. Our morphological findings indicate the probability that in the adrenal cortex, as in the liver, the primary lesion occurs in the capillary system. On this hypothesis, the different degrees of severity of peliosis in the adrenal cortex could be the consequence of varying degrees of estrogenic stimulation.

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