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Original article

A quantitative histological study of adrenal development during the perinatal period in intact and hypophysectomized pigs

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Summary — Adrenal growth was studied between D70 of gestation (D0 : day of mating) and D6 after birth in 33 fetuses and 11 new-born pigs of the Large White breed. Volume, height and mean surface, were estimated by zone, as well as cell number, cell size and nucleocytoplasmic ratio. The volume increased exponentially. In the cortex it was 4.0 mm^3, 7.2 mm^3, 10.5 mm^3, 33.6 mm^3, 55.3 mm^3 at D70, D94, D106, D113 and D1-D6, respectively, after birth. In the medulla, the volume was 4.0 mm^3, 6.3 mm^3, 6.9 mm^3, 9,6 mm^3 and 20.6 mm^3 at the same stages. This evolution was due to predominant longitudinal stretching between D94 and D106, then thickening, as shown by the relative variations of the height and the surface. The growth of the cortex corresponded to hyperplasia associated with hypertrophy after D110. In the medulla, hyperplasia predominated until D110; this was followed by hypertrophic phase. Twelve fetuses hypophysectomized at D44 or at D55 were recovered at D94, D106 and D112 at least 50 days after surgery. Hypophysectomy did not affect medullar development (volume and number of cells) but inhibited very significantly the growth of the cortical zone, particularly that of the fasciculata.

pig — fetus — newborn — adrenals — volume — surface — cell number

Résumé — Données quantitatives histologiques du développement des surrénales pendant la période périnatale, chez le porc normal et hypophysectomisé. *La croissance des surrénales du porc de race Large White a été étudiée entre G70, (G0 étant le jour de la saillie), et le 6e jour* post partum *chez 33 fœtus et 11 nouveau-nés. Le volume, la hauteur et la section moyens, le nombre de cellules, leur taille et le rapport nucléocytoplasmique ont été estimés par zone. Le volume des différentes parties a augmenté de façon exponentielle. Pour le cortex, il est respectivement égal à 4,0 mm^3, 7,2 mm^3, 10,5 mm^3, 33,6 mm^3 et 55,3 mm^3 à G70, G94, G106, G113, et 1 à 6 jours après la naissance. Pour la médulla, le volume est égal à 4,0 mm^3, 6,3 mm^3, 6,9 mm^3, 9,6 mm^3 et 20,6 mm^3 aux mêmes âges. Cet accroissement est dû à un allongement prédominant entre G94 et G106, puis un épaississement, comme le montrent les variations relatives de la hauteur et de la surface. La croissance du cortex correspond à une hyperplasie associée à une hypertrophie après G110. Dans la médulla, une hyperplasie prédomine jusqu'à G110, elle est suivie d'une phase d'hypertrophie. 12 fœtus, hypophysectomisés à G44 ou à G55 ont été récupérés à G94, G106 et G112, au moins 50 jours après l'opération. L'hypophysectomie n'a eu aucun effet sur le développement de la médulla (volume, nombre de cellules) mais elle a inhibé de façon très significative la croissance de la zone corticale, essentiellement, celle de la zone fasciculée.*

porc — fœtus — nouveau-né — surrénales — volume — surface — nombre de cellules

Introduction

In the domestic pig, birth follows a period during which cortisol or corticosteroid levels increase exponentially in the fetal plasma (Dvorak, 1972; Fèvre, 1975; Randall, 1983). This increased production of corticosteroids is due to a maturation of cortical cells and to the growth of the cortical zone (Lohse and First, 1981). The formation of the adrenals (Flint, 1900; Wiesel, 1901; Whitehead, 1903; Weymann, 1922-1923; Katznelson, 1965; Bielanska-Osuchowska, 1976) and their organization during fetal life (Katznelson, 1966; Gutte *et al.*, 1986) has already been described in pigs. Weight variations of the whole gland have also been reported (Ullrey *et al.*, 1965; Bosc, 1973; Lohse and First, 1981), but the development of the 2 zones of the adrenals is not well known, particularly during the perinatal period. The aim of this study is to provide data on this development as well as to compare normal and hypophysectomized fetuses. It is already known that hypophysectomy of the pig fetus inhibits the final growth of the adrenals normally observed during the last days of gestation (Bosc *et al.*, 1974).

Materials and Methods

Thirty-three normal fetuses were obtained by caesarian section or after slaughter of the mothers (Large White sows) between 70 and 113 d of gestation (D0 : day of mating or artificial insemination); 20 were taken before D100 and 13 between D100 and D113. Eleven normal new-borns were also obtained at the ages of 1 (n = 3), 3 (n = 6) and 6 (n = 2) days, and for convenience their ages were expressed in days of gestation, *i.e.* D115, D117 and D120 respectively. Twelve fetuses, hypophysectomized as previously described (Bosc *et al.*, 1974) on D44 or D55, were recovered respectively on D94-D95 (n = 4) and on D106-D107 (n=5) or D112-D113 (n=3). The absence of the pituitary was confirmed at autopsy and undicated by the lack of prolactin in the plasma (Ravault *et al.*, 1982).

Following dissection, the adrenals were fixed in acetic Bouin Hollande solution and embedded in paraffin; in most cases they were previously weighed. After cutting in 10 μm sections, they were stained by a trichrome (Gabe, 1968) containing hematoxylin of Groat, erythrosin, orange G and toluidine blue. For cortex and medulla, the volume, height, mean surface, number of cells, nuclear surface and nucleocytoplasmic ratio were determined.

The total volume of the gland (Vt) and volumes of the medulla (Vm) and the cortex ($Vc = Vt - Vm$) were estimated from elementary volumes delimited between 2 sections. Each elementary volume was calculated according to the formula : $V\ (mm^3) = 1/3\ h\ (S + S' + \sqrt{SS'})$, where S and S' are the respective surfaces of the 2 sections delimiting it and h (mm) is the height between them. The surfaces were measured with a planimeter (Leitz microscope coupled with a table of integration).

A preliminary study was made to establish the minimum number of elementary volumes necessary to evaluate with precision Vt, Vm or Vc. For this study, 5 pairs of adrenals were taken from 4 control and 1 hypophysectomized animal at D70, D94, D103, D107 (hypophysectomized) and D117. For each adrenal, a reference volume was determined with a number of elementary volumes varying between 67 and 234 according to adrenal size. The volume was recalculated by successive reduction of the number of elementary volumes and all volumes obtained were compared to the reference volume. This procedure indicated that, at the moment of slicing, and to limit the variation of the volume to less than 5%, it was sufficient to keep 1 section out of 30.

Adrenals were characterized by 3 volumes — Vt, Vc and Vm — without taking into account shrinkage (mean ± SD, 0.47 ± 0.08) due to the fixation. Shrinkage was estimated by the total volume divided by the weight for each adrenal; these two parameters were highly correlated (r = 0,97). We also calculated the total height of the gland according to the number of sections and the mean surface. This mean surface represented an average of all the section areas used for the volume calculation.

The number of cells per adrenal was estimated for the fasciculata and medulla zones of fetuses of at least 90 d. At this stage, the cortex and medulla are well differentiated, (Katznelson, 1966; Bielanska-Osuchowska, 1976; Gutte *et al.*, 1986). Counting was performed on 5 μm thick sections from 15 different areas within each third of the gland (reticle Zeiss with 25 points). The number of cells was equivalent to the numbered nuclei, corrected by taking into account the size of nuclei and section thickness according to Aherne (Solari, 1977). Thereafter, the nucleocytoplasmic ratio (NCR) was estimated by the quotient of the number of points on the nuclei divided by the sum of the points on the nuclei and the cytoplasm. The points on the conjunctive tissue and on the vessels were also noted.

The surfaces of nuclear sections were also measured in 3 fetuses (90, 100, 110 d-old) and 1 new-born (3 d-old). Analysis of the surfaces, showed 1 population of cells per zone and per stage. According to these measurements, cell volume could be estimated from the nuclear surfaces, assuming that the nucleus was a sphere.

All parameters were studied relative to age and reproduced on graphs using curve smoothing procedures (Cleveland, 1979; Becker and Chambers, 1984). The correlations were calculated after Pearson (Vessereau, 1960); the estimated growth rates were calculated by taking into account the exponential nature of the data by an adjustment according to the formula $y = ae^{bx}$. Variance analysis (Vessereau, 1960) was used to compare normal and hypophysectomized fetuses of the same age and to compare different ages in the same type of animal. All calculations were made without consideration of the sex of the animal ($P > 0.05$) or whether the left or right adrenals were utilised ($r = 0.96$ for the weight).

Results

In control animals, adrenal weight and volume increased exponentially during the whole period. This increase was more pronounced after D106—D107 (Fig. 1). The weight of fetuses also showed exponential growth but the increase was

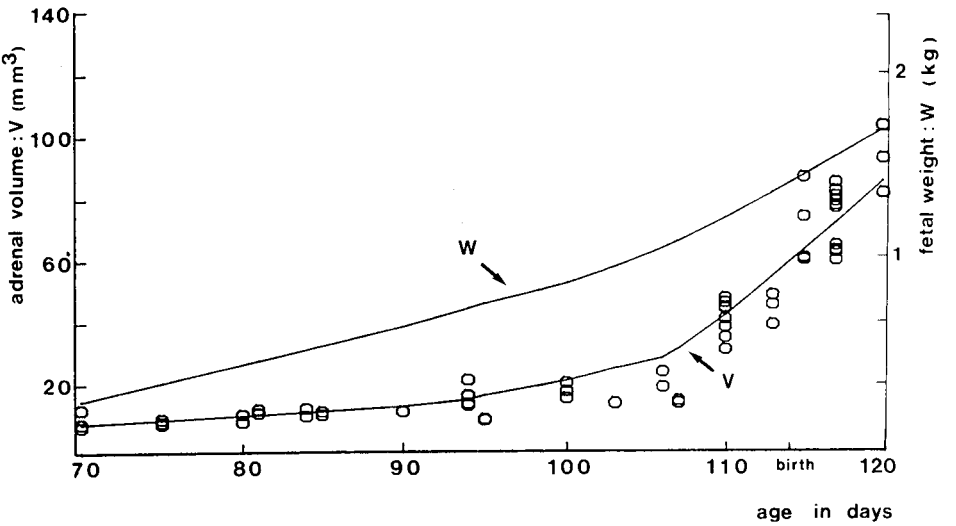

Fig. 1. Variations of fetal weight (W : kg) and adrenal total volume (V : mm^3), according to the gestational age in the domestic pig. These curves have been obtained by a smoothing procedure. Individual volume (O) of each adrenal is also represented. For the new-born, birth date is equivalent to D114.

less than that of adrenal weight ($r = 0.80$) (Fig. 1). At all stages, the cortex and medulla were well delineated, but many cortical cells grouped in rows were seen in the medulla between D70 and D80. The volume of the medulla had a daily rate of growth of 0.10, 0.05 and 0.47 respectively, between D70 and D94, D94 and D106, D106 and D112 (Table I). In the same periods, the rates of cortical growth were 0.13, 0.28 and 3.30, respectively (Table I). Variations of the relative volumes of the medulla (Vm/Vt) and of the cortex (Vc/Vt) confirmed the differences in growth rates between the 2 areas (Fig. 2). The cortex developed faster than the medulla after D94, being further accentuated after D106 (Figs. 2 and 3).

Analysis of the height (h) and mean surface (S) of the adrenals showed several stages of development for the volumes of the 2 zones. For the medulla, the ratio $h/\sqrt{S}$ was not modified significantly until D100; it increased from D100 to D106 and decreased thereafter. This ratio followed a similar pattern for the cortex, but its increase between D100 and D106 was less pronounced than in the medulla. The general evolution indicated a longitudinal stretching equivalent to thickening until D100; this was quicker between D100 and D106 before declining (Fig. 4).

A comparison of the data from 4 age groups confirmed these patterns (Table I). Adrenal weight increased significantly between the different ages ($P < 0.05$).

Table I. Weight, cortical and medulla volumes of adrenals in control and hypophysectomized fetuses at 4 stages of gestation.

Animals *Type*	*Age*	*Adrenal weight (mg)*	*Cortical volume (mm^3)*	*Medulla volume (mm^3)*
Control	D94—D95	25.6 ± 7.1^{a} (3)	7.2 ± 3.2^{ag} (8)	6.3 ± 1.4 (8)
H *		18.3 ± 4.6^{ef} (4)	2.1 ± 0.8^{efg} (2)	3.4 ± 0.1^{f} (2)
Control	D106—D107	43.0 ± 7.9^{ab} (6)	10.5 ± 0.6^{abh} (4)	6.9 ± 0.9^{a} (4)
H **		39.1 ± 18.2^{e} (10)	4.4 ± 0.6^{eh} (6)	8.2 ± 2.0^{ef} (6)
Control	D112—D113	121.0 ± 11.3^{bc} (4)	33.6 ± 4.4^{bdj} (4)	9.6 ± 1.6^{ab} (4)
H **		52.3 ± 14.0^{f} (6)	5.7 ± 0.9^{fi} (5)	9.9 ± 2.8^{e} (5)
Control	D115—D120	150.0 ± 26.1^{c} (21)	55.3 ± 11.4^{d} (17)	20.6 ± 2.8^{b} (17)

H = Hypophysectomized : at D44* and D55**; D0 = Day of mating; D 115 = Equivalent to D1 after birth; Values are mean ± SD *(N)*. Comparisons have been made 2 x 2 between control and hypophysectomized fetuses at a same age or between ages for each type of animals : $^{a, c, e, g} P < 0.05$; $^{b, d, f, h, i} P < 0.01$.

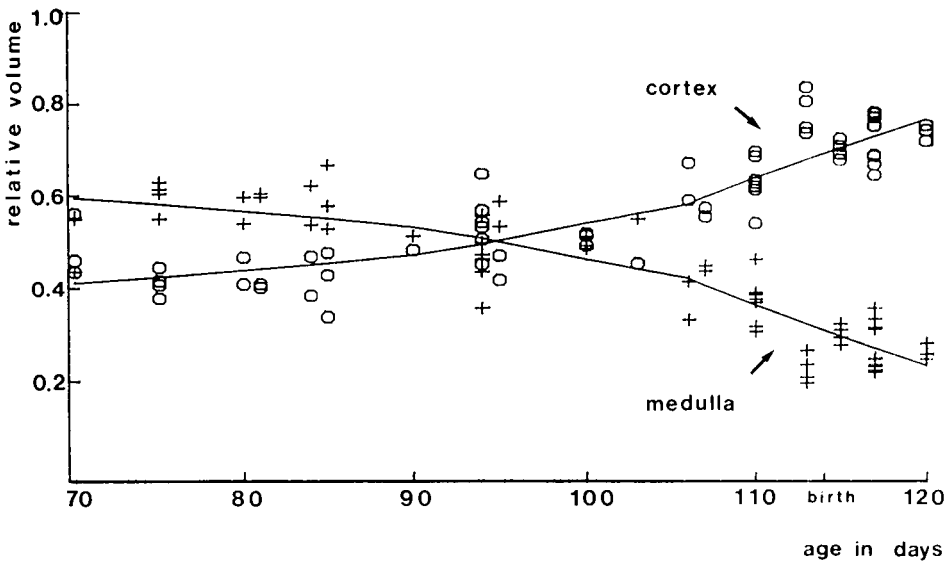

Fig. 2. Relative volume of the cortex (Vc/Vt : 0), and of the medulla (Vm/Vt : +), according to the gestational age in the domestic pig. Their smoothed curves are also represented. Vc, Vm and Vt are the volume of cortex, medulla and the whole gland respectively.

Increase of the medulla and cortical volumes was low between D94 and D106 ($P < 0.05$); it was higher between D106 and D112 in the medulla ($P < 0.05$), and cortex ($P < 0.01$), and between D112 and D115-D120 for the 2 parts ($P < 0.01$).

The area of the cell nuclei was modified between D90 and D117 in the cortex ($P < 0.05$), but not in the medulla ($P > 0.05$). This variation was due to the new-born (D117), whose nuclei were larger than those of the fetuses ($P < 0.05$) (Table II). At D80 the nuclei, stained with hematoxylin, were often lengthened and clear in the fasciculata zone (cortex internal part), but more circular and dark

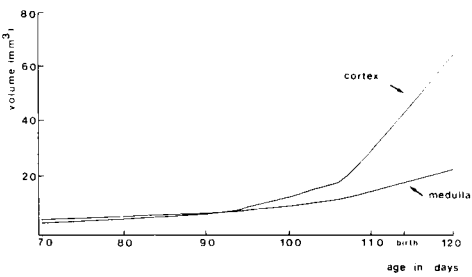

Fig. 3. Smoothing curves of cortical and medulla volumes (mm^3) according to the gestational age in the domestic pig.

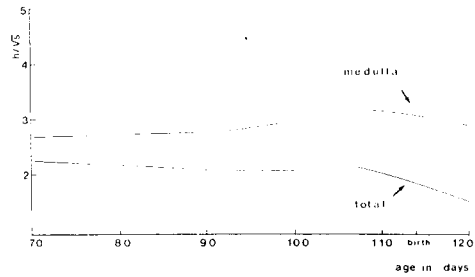

Fig. 4. Variations of the ratio of the height (h), by the square root of the mean section (s), according to the gestational age in each adrenal zone, in the domestic pig. These curves have been obtained by a smoothing procedure.

Table II. Nuclear surface, nucleocytoplasmic ratio, cell volume and total number of cells at different ages, during gestation and after birth.

Age	*Nuclear surface (μm^2)*		*Nucleocytoplasmic ratio*		*Cell volume (μm^3)*		*Cell number x 10^3*	
	Cortex	*Medulla*	*Cortex*	*Medulla*	*Cortex*	*Medulla*	*Cortex*	*Medulla*
D90	23.2 ± 4.0^{ab}	26.2 ± 6.1^{b}	0.21 ± 0.06	0.27 ± 0.10	402	361	4737	4906
D100	23.9 ± 3.8^{b}	26.5 ± 5.7^{b}	0.22 ± 0.07	0.24 ± 0.10	374	419	7823	7505
D110	24.3 ± 4.7	24.2 ± 6.1	0.19 ± 0.10	0.26 ± 0.10	405	264	19127	13025
D117	29.3 ± 6.0^{ab}	25.4 ± 6.0^{b}	0.18 ± 0.06	0.22 ± 0.10	778	411	37268	13782

D0 = Day of mating. D117 = Equivalent to D3 after birth.
The nuclei surfaces were measured on 2 x 100 nuclei by zone and by age, comparisons were made by zone (^{a} = $P < 0.01$) or between zones at a same age (^{b} = $P < 0.01$).
The number of cells and the nucleocytoplasmic (NCR) ratio were estimated by zone on 2, 3, 8 and 6 adrenals respectively, at D90, D100, D110 and D117. The NCR was higher in the medulla than in the cortex ($P < 0.05$). The cells volumes were calculated according to the nuclei volume and the corresponding nucleocytoplasmic ratio.

in the glomerulosa zone. In the medulla, the nuclei were round and clear. From D90, the cytoplasm was more stained in the cortex, the conjunctive tissue was stained by toluidine blue and the vascular system, stained by orange G, was more developed in the medulla.

The nucleocytoplasmic ratio was variable, but always higher in the medulla than in the cortex ($P < 0.05$). It was lower in the 2 zones at D117 in comparison with D90, D100 and D110 ($P > 0.05$). The mean volume of the cortical cells did not vary between D90 and D110, though an increase of 92.1% occurred between D110 and D117. In the medulla, the mean cellular volume fluctuated around 364 μm^3 between D90 and D117; the lowest value was obtained at D110. Due to the increase of the volume of the two zones and the variation of mean cell volume, the total number of cells tripled in the medulla and octupled in the cortex during the studied period (D90—D117) (Table II).

In the hypophysectomized fetuses, adrenal weight doubled between D94 and D106 ($P < 0.05$) and tripled between D94 and D112. Increases were also observed for the cortical and medulla volumes ($P < 0.05$ and $P < 0.01$ according to the case), but they were low between D106 and D112 (Table I). However, in hypophysectomized fetuses, the cortical volume was lower than in control fetuses at the 3 studied ages ($P < 0.05$ at D94; $P < 0.01$ at D106 and D112) (Table I). Fifty days after surgery, the fasciculata zone was considerably reduced while the glomerulosa and medulla zones did not appear to be affected. For these reasons, counting in the fasciculata zone was impossible in most of the fetuses.

Discussion

The results show that the cortical and medulla zones of the pig adrenal have

exponential growth during the perinatal period and that the fetal hypophysis controls adrenal cortex growth. In the cortex, growth rate is low before D94; it accelerates and becomes explosive after D106 (Fig. 2). As the cortex has a greater rate of development than the medulla, it occupies most of the adrenal just before and after birth. Between D94 and D106 lengthening is quicker than thickening, which predominates later. Thus, a median section of the fetal adrenal does not totally represent the growth of the whole gland as admitted in previous studies of pigs (Lohse and First, 1981) or sheep (Nathanielsz *et al.*, 1972).

Growth of the cortex corresponds to a cellular hyperplasia which is associated with a cellular hypertrophy after D110. Growth of the medulla is due to cell multiplication until D110; afterwards, it results from a phase of hypotrophy followed by a phase of hypertrophy (Table II). Thus, in the pig, the perinatal period is characterized by a cellular hypertrophy phase in the 2 adrenal parts. However, for the whole gland, there is a hyperplasic preponderance due to the development of the cortex. Thus, between D90 and D117, the whole volume has a mean daily increase of 5.2.% which corresponds to an increase in cell number of 9.1% in the cortex as compared with 3.7% in the medulla.

The number of cells determined by zone and by age corresponds to 1 cellular type. They are smaller than those estimated in a previous study (Lohse and First, 1981), in which all the gland components, such as the vascular system and the conjunctive tissue, had not been accounted for. When these components are omitted, the numbers found from our data are in agreement with the previous results, which had been overestimated.

In our study, as in that of Lohse and First (1981), the last phase of development emphasizes the cortex and particularly the zona fasciculata. It corresponds to an increased production of corticosteroids (Dvorak, 1972; Fèvre, 1975) and of fetal cortisol (Randall, 1983). Growth of the cortex is certainly one of the main factors which could explain this characteristic feature of the perinatal period, even if it is accompanied by intracellular biochemical modifications in pigs (Lohse and First, 1981) and fetal sheep (Durand, 1987). The growth of the cortical zone depends on hypophyseal secretions during the second half of gestation (Table I). As in sheep, hypophysectomy does not affect the growth of the medulla and glomerulosa zones in pigs during this period (Liggins, 1969; Robinson *et al.*, 1983). It is not known if hypophysectomy of fetal pigs influences the functional activity of the medulla zone. It is likely, because corticosteroids are known to exert regulatory influences on several of the cathecholamine biosynthetic enzymes of the medulla (Weiner, 1975).

The qualitative characteristics observed in this study correspond to those described previously (Katznelson, 1966; Gutte *et al.*, 1986). These authors emphasize once again, the important development of the fasciculata zone, which is particularly rich in lipidic cells during the last week of gestation.

In conclusion, the cortex and the medulla of the pig adrenals develop exponentially during the perinatal period. However, they differ in growth rate and in their controls due to pituitary factors. These differences reflect 2 tissues which have different embryonic origins (Wiesel, 1901; Whitehead, 1903; Katznelson, 1966; Bielanska-Osuchowska, 1976).

Acknowledgments

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