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Isolation and electrophoretic identification of exogenous histone from rat blood plasma

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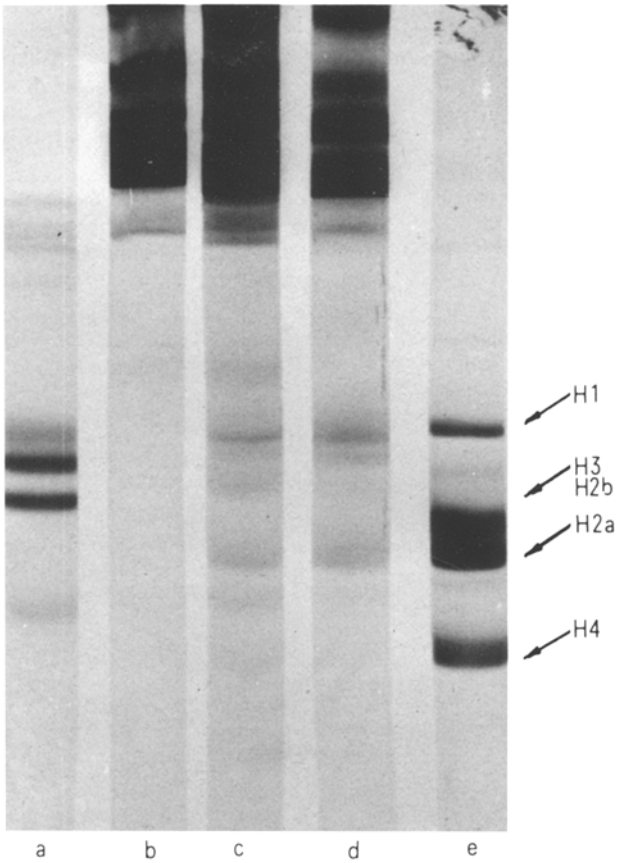
Summary. Histone fractions H1, (H2b+H2a) and H4 were isolated from rat blood plasma and electrophoretically identified 5 min after i.v. exogenous histone application.

The action of various chemical, mechanical, termic and toxic agents might result in cell destruction. Furthermore in case of structural and metabolic nuclear disturbance, some of the nuclear proteins-histones might leave chromatin and pass to other nuclear or cytoplasmic structures, or leave the cell[1-3]. Our previous studies have shown that histone proteins can be isolated from both normal and tumour rat blood plasma[4]. The electrophoretic pattern of isolated exogenous histones from rat blood plasma and the influence of globin fractions with the same electrophoretic mobility are the subject of the present study, carried out by the method previously employed[4].



Polyacrylamid gel electrophoresis of: *a* Globin; *b* blood plasma before i.v. application of exogenous histones; *c* untreated plasma after application; *d* treated plasma after application; *e* whole histone.

Material and methods. Wistar rats were i.v. injected 5 mg/100 g b. wt whole histone dissolved in 0.5 ml of 0.9% NaCl. 5 min later a blood sample was taken and centrifuged at 4 °C. The plasma so obtained was precipitated with trichloracetic acid to a final concentration of 18%. The precipitate was homogenized with 10 vol. of 0.25 M sucrose, 0.1 M Tris-HCl pH 7.4 and 0.003 M $CaCl_2$. Histones were extracted twice by homogenization of the sediment with 0.25 M H_2SO_4. The combined supernatants were clarified by filtration and histones were precipitated with 6 vol. of ethanol for a night at − 10 °C. The sediment thus obtained was washed and dissolved in 0.9 N CH_3COOH and 15% sucrose. Blood plasma samples obtained from each rat before and after histone injecting which were not treated in the aforesaid manner, served as controls. Whole histone was extracted according to Spelsberg and Hnilica[5] from liver chromatin obtained according to Tsanev and Russev[6]. Globin was obtained according to Teale[7]. Acrylamide gel electrophoresis was carried out according to Panyim and Chalkley[8].

Results and discussion. 5 min after an exogenous whole histone application histone fractions H1, (H2b+H2a), H4 are to be found in plasma corresponding to the standard histones (figure, c and e). Arginine-rich histones H3 only are missing, being probably adsorbed on erythrocyte membranes; passing over to subcellular structures is also probable[1,9]. Globin bands can be seen among histone fractions as a result of haemolysis due to partial erythrocyte destruction under the exogenous histone influence and dissociation of globin from haem because of acid buffer of gel electrophoresis (figure, a-c). Globin contaminations are eliminated after the above-mentioned plasma treatment and fractions H1, (H2b+H2a) become sharply outlined on the electrophoretic pattern (figure, a, c and d). However fraction H4 is less apparent in this case, probably due to partial loss at some stages of treatment (figure, c and d). Longer exposition was not carried out, since exogenous histones are eliminated up to 60–70% in 30 min and remain only 6–25% in plasma by the 6th h after application[10]. Our results demonstrate that histones present in rat blood

plasma can be isolated and electrophoretically identified with some changes in arginine-rich H3-H4 fractions, and this confirmed our previous study[4].

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Progesterone receptors in the foetal uterus of guinea-pig: Its stimulation after oestradiol treatment

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Summary. This paper shows for the first time the presence of progesterone receptors in the foetal guinea-pig uterus, as well as the stimulation of progesterone receptors in foetal uterus in animals treated with oestradiol.

Previous studies in this laboratory have demonstrated the presence of oestradiol receptors in the foetal uterus of guinea-pig[1]. These receptors appear in this foetal tissue at an early age of gestation, increase during foetal development and decrease after birth[2]. In this paper, the existence of progesterone receptors in the same foetal tissue, at the end of gestation, and its stimulation in oestradiol-treated animals are presented.

Foetuses of Hartley Albino guinea-pigs (50 days to the end of gestation) were used. Females and males were mated for 24 h, consequently the days of gestation were established with an error of ±24 h. Experiments were carried out after incubation of ^{3}H-progesterone (s.a.: 55 Ci/mmole, NEN Chemical, GmbH, Frankfurt, GFR), with the total cell (cell suspensions) or with the cytosol fraction in Krebs Henseleit buffer. The subcellular fractionation of the foetal uterus was carried out according to the method of Chauveau et al.[3]. The cytosol fraction was obtained by centrifugation at 250,000× g. Purified nuclei were extracted successively with the following solutions a) 0.1 M TRIS.HCl - 0.0015 M EDTA (0.1 M TRIS); b) 0.3 M NaCl - 0.01 TRIS.HCl (0.3 M NaCl) and c) 1 M NaCl - 0.01 M TRIS.HCl (1 M NaCl). Sucrose density gradients were carried out in 5-20% w/v sucrose solutions containing 0.012 M thioglycerol. Centrifugations were carried out at 250,000× g at 2 °C for 16 h. Proteins were measured according to the method of Lowry et al.[4]. Specific binding was determined after incubation of the total cell or the cytosol fraction with ^{3}H-progesterone alone or with a 100-fold excess of unlabelled progesterone, and calculated by the difference in binding between these 2 incubations as determined using the dextran charcoal method[5]. Equilibrium constants were measured by the Scatchard method[6].

Figure 1 shows an example of a Scatchard plot for ^{3}H-progesterone binding in the cytosol fraction of the fetal guinea-pig uterus. The dissociation constant is ($Kd_{4°}$) 3.6 ± 10^{-9} M and the number of sites $n = 180 \pm 28 \times 10^{-15}$ moles/mg protein. Progesterone and the synthetic progestagen R-5020 (17,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione) compete significantly in the formation of the ^{3}H-progesterone-protein complex, but oestradiol, oestrone or cortisol have no effect. The synthetic progestagen ^{3}H-R-5020 was also found to bind specifically in the cytosol fraction of the uterus.

The analysis by ultracentrifugation in sucrose gradient shows the presence of a component with a sedimentation coeficient of 6-7 S and another with 4-4.5 S. It is interesting to note that similar components were found for the ^{3}H-progesterone complex in human endometrium[7]. The specific protein which binds progesterone in the foetal uterus is not a contamination of foetal plasma protein, which also binds progesterone[8], because in the cytosol fraction of the foetal uterus which was heated for 1 h at 37 °C before being incubated with ^{3}H-progesterone (4×10^{-9} M) at 4 °C for 4 h, it was observed that 85-95% of the specific binding sites of progesterone were destroyed. No effect of temperature was observed in the formation of the ^{3}H-progesterone protein

Table 1. Effect of oestradiol on the weight of foetal uterus of guinea-pig after injection of oestradiol to the mother

Days of gestation	Control animals (in mg per uterus)	Treated animals
56-57	40.0±3	67.0±7.0
58-59	41.5±4.5	80.7±6.6
64-65	93 ±3	167 ±13.1

Each pregnant guinea-pig received 1 mg/kg/day of oestradiol. The values correspond to the data obtained in 4 uteri of 3 experiments (means ± SD).

Table 2. Effect of oestradiol on the quantity of progesterone receptors in the foetal guinea-pig uterus

Days of gestation	Subcellular fraction	Specific progesterone binding sites (fmoles/mg protein) Controls	Treated animals
48-52[a]	Cytosol	29	97
60-65[a]	Cytosol	66	407
58-59[b]	Cytosol	45	175
	Nuclear extracts		
	0.1 M Tris	ND	15
	0.3 M NaCl	ND	12
	1 M NaCl	3	48

Pregnant guinea-pigs were treated as indicated in Table 1.
[a] Incubation of the foetal cytosol fraction with ^{3}H-progesterone (4×10^{-9} M) with or without a 100-fold excess of unlabelled progesterone 4 h at 4 °C. [b] Incubation of cell suspensions with ^{3}H-progesterone (8×10^{-9} M) with or without a 100-fold excess of unlabelled progesterone 15 min at 37 °C. The data represent the average values with 4 uteri of 3 experiments.
ND, not detectable.