peared earlier in the migration areas, but the catalase activity lingers longer in the intermediate area as compared to untreated rats.

The histochemical changes reported in this study showed a delay in the appearance of catalase-containing and catecholaminergic fluorescent cells in the mediobasal hypothalamus. The time lost at the beginning of their pubertal development is made up by a faster displacement from the median eminence towards the arcuate nucleus. This is in contrast to the results of prolactin administration[29], which delayed total fluorescence of catecholaminergic cells, but also the expression of catalase activity during the displacement.

In terms of the model proposed by Tresquerres et al.[20] these results could be explained by the presence of a tonic center which has an effect on hypothalamic development. Although the damaging of the cyclic center causes a delay in the maturation of the hypothalamus it can in fact be overcome by the plasticity of the system and by the action of the tonic center.

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Dense core vesicles during photoreceptor development

J.A. Armengol, F. Prada, A. Quesada and J.M. Génis-Gálvez[1]

Instituto de Biologia del Desarrollo, Departamento de Anatomia, Facultad de Medicina, Avda. Sánchez Pizjuan 4, Sevilla (Spain),
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Summary. The presence of dense core vesicles in the terminal expansions of photoreceptors in development is decribed in the chick embryo retina, from the 16th to the 18th day of incubation.
Key words. Chick embryo; retina; photoreceptor; dense core vesicles.

In the course of our studies on the histogenesis of the chick retina, we have detected various vesicle populations at the synaptic terminals of developing photoreceptors. Although these vesicles (coated vesicles, dense core vesicles and clear vesicles), have been described in the adult retina[2-4], the simultaneous existence of clear and dense core vesicles, together in the same synaptic terminal, has never been observed in normal animals.

We have used 168 retinas of chick embryos at various days of incubation (10th–20th) (table 1). After enucleation, the retina was dissected and fixed by immersion in 3% glutaraldehyde, 1% formaldehyde and 0.5% acrolein in 0.1 M sodium cacodylate buffer (pH 7.2), and postfixed in 2% osmium tetroxide, dehydrated in a graded series of acetones, and stained in block (0.5% uranyl acetate in 70% acetone for 1 or 2 h). The pieces of retina were embedded in Araldyte CY 212 (Durcupan). Ultrathin sections (600 A) were stained with uranyl acetate and lead citrate. A Jeol 100C electron microscope was used for observation at 80kV.

Table 1. Relationships of the embryos and retinas of various stages used

Day of incubation	Stage	No. embryos	No. retinas
10	HH-36	11	13
11	HH-37	6	9
12	HH-38	7	10
13	HH-39	7	14
14	HH-40	10	16
15	HH-41	10	20
16	HH-42	10	20
17	HH-43	12	20
18	HH-44	10	20
19	HH-45	8	16
20	HH-46	5	10
		96	168

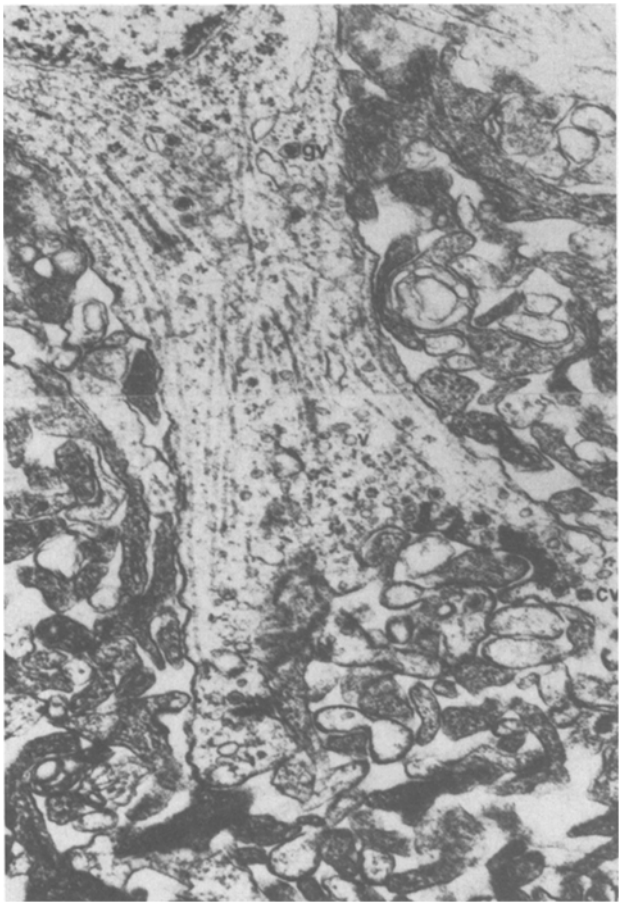

Figure 1. 18-day-old chick embryo retina. Coated vesicles (cv), clear vesicles (v) and dense core vesicles (gv) can be observed in the synaptic terminal of the photoreceptor. × 25,000.

The outer plexiform layer of the embryonic retina shows its early organization towards the 12th–14th day of incubation[5,6], the stage in which the first synaptic ribbons can already be recognized in the synaptic terminals of the photoreceptors. From the 16th day of incubation, three vesicle populations are detected in the terminals of these photoreceptors in development (table 2); this characteristic becomes very evident by the 18th day of incubation. These vesicle populations are composed of coated vesicles, dense core vesicles of various sizes, and clear vesicles (fig. 1). In subsequent days of development (19th–20th days of incubation), the loss of the dense core vesicles from the pedicles of the photoreceptors is observed (table 2); the photoreceptors acquiring a morphology which is very similar to that of the cells of the adult retina (fig. 2).

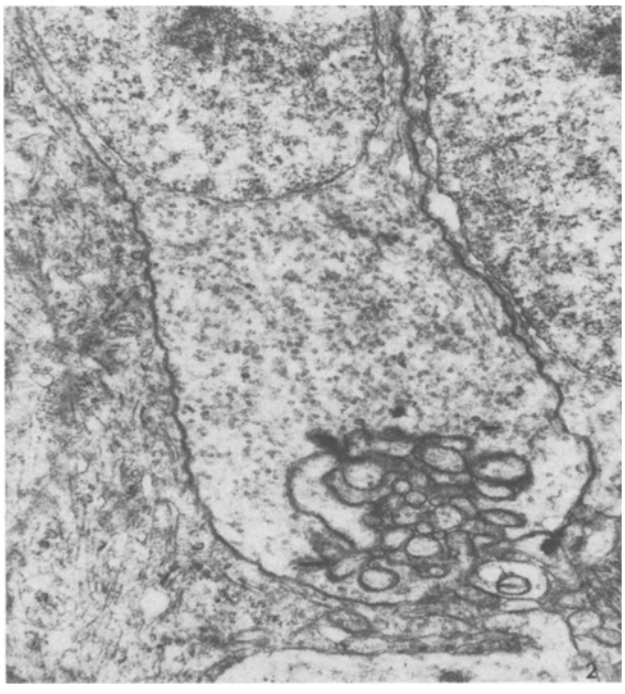

Figure 2. 20-day-old chick embryo retina. Note the absence of dense core vesicles in the photoreceptor. × 18,500.

The coated vesicles have been described as endocytic elements which can intervene in the uptake of the released neurotransmitter[7]. The adult photoreceptors possess, in addition to coated vesicles, clear vesicles which store their neurotransmitter[8], and only in experimental conditions of continuous exposure to light has the presence of dense core vesicles been observed in the spherules of the rods[3,4]. Our results show the co-existence of clear vesicles and dense core vesicles in the synaptic terminals of the photoreceptors in the later phases of development (table 2). The presence of the dense core vesicles in these synaptic terminals coincides with the invagination of the post-synaptic expansions in the interior of the synaptic terminal of the photo-receptors in development; disappearing once these synapses have a morphology very similar to that of the photoreceptors of the adult retina. Our observations agree with those of Meller and Tetzlaff[9], in locating this last phase of the maturation of the photoreceptors towards the 19th day of incubation. The presence of the dense core vesicles only during the process of invagination of the future synaptic triads leads us to think of the possibility that the content of these vesicles could play a role in stimulating this process. From this there arises an interesting hypothesis, which will have to be investigated from the ultracytochemical and pharmacological points of view.

Table 2. Shows the various time of appearance of the various populations of vesicles. The mean value of the synaptic invagination has been taken in each case for 100 synaptic terminals of totally vertical sections, including 75% cones and 25% rods

	10	11	12	13	14	15	16	17	18	19	20	
Clear vesicles	++*	++	++	++	+++	+++	+++	+++	+++	+++	+++	rods
				++	+++	+++	+++	+++	+++	+++	+++	cones
Coated vesicles	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	rods
				+++	+++	+++	+++	+++	+++	+++	+++	cones
Dense core vesicles	–	–	–	–	–	±	++	+++	+++	+	–	rods
				–	–	±	+	+++	+++	+	–	cones
Postsynaptic invagination	0	0	0	0**	0.40	0.52	0.70	0.86	1.06	1.46	1.80	

Mean values μm; * pleomorphic vesicles; ** approximately 0.10–0.15 in some cases; +, 1–5 vesicles per section; ++, 6–15 vesicles per section; +++, more of 15 vesicles per section.

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Stimulation of histamine synthesis in rat mast cells by compound 48/80 in vitro

A.M. Rothschild and I.C. Fortunato

Department of Pharmacology, School of Medicine of Ribeirão Preto, USP, Ribeirão Preto (SP. 14100, Brazil), 17 October 1983

Summary. The histamine content of rat peritoneal fluid cells is doubled within 20 min by 0.5 µg/ml of compound 48/80. Histamine catabolism inhibitors do not reproduce this effect; cells pre-incubated with α-fluoromethylhistidine are unresponsive to compound 48/80 which therefore activates pre-formed histidine decarboxylase rather than 'inducing' it. Non-mast cells showed no change after treatment with compound 48/80.
Key words. Rat mast cells; histamine synthesis; compound 48/80.

Previous work[1] has shown that rats given relatively high doses of adrenaline present a rapid rise of up to 50% in skin and lung histamine, attributed to the stimulation of histamine synthesis in tissue mast cells. Adrenaline has also been shown to enhance mast cell ability to act on substituted arginine or tyrosine esters[2] and to activate rat plasma kallikrein[3]. Since mast cells exposed to compound 48/80 presented similiar activities, the present work was designed to investigate whether, like adrenaline, compound 48/80 stimulates mast cells to synthesize histamine rapidly in vitro.

Materials and methods. Peritoneal fluid cells were harvested from Wistar rats and incubated in Krebs Ringer phosphate buffer at 37 °C under air as previously described[2]. Histamine was assayed fluorimetrically[4] omitting the purification procedure[5]. Mast cells were separated by centrifugation into 38% bovine serum albumin[6,2]. Compound 48/80, aminoguanidine, histamine diphosphate and o-phtaldialdehyde were obtained from Sigma Chem. Co., St. Louis, USA. Compound SK+F 91488 (S- 4-(dimethylaminobutyl) isothiourea) and α-fluoromethylhistidine were gifts from Dr M.E. Parsons; Smith, Kline and French, Welwyn Garden, Herts, U.K. and Dr J. Kollonitsch; Merck, Sharp and Dohme, West Point, Pa, USA, respectively.

Results. Table 1 shows that incubation of rat peritoneal fluid cells with compound 48/80 leads to an increase in both free and total histamine of the incubates; it is clear, therefore, that the percentual increase in the free form of the amine cannot, as is often stated, be solely due to the release of pre-formed, cell-bound histamine. Although presenting fairly pronounced variability from one animal to another, these findings were quite reproducible; they appeared somewhat as a surprise, in view of the lack of reference to similar observations in the numerous reports available on the effects of compound 48/80 on rat peritoneal fluid cells[7,8]. The data presented in figure 1 offer an explanation for this lack; they show that in contrast to histamine release, the build-up of histamine in compound 48/80-incubates is a rather sluggish process which only acquires momentum after 5–10 min, i.e. long after the period required for maximal histamine release has elapsed. Since most previous studies using compound 48/80 as a histamine releaser from rat peritoneal fluid cells employed incubation periods of less than 10 min, it is not difficult to understand why histamine enrichment of such incubates was overlooked.

It appeared to be important to investigate whether histamine accumulation due to compound 48/80 could be due to an arrest of the eventual destruction of the amine during prolonged incubation. Table 2 shows this to be improbable, firstly

Table 1. Increases in histamine in rat peritoneal fluid cell suspensions exposed to compound 48/80 in vitro

Treatment	Histamine (µg/flask) In medium (free H)	percent of total	In cells	Total
Buffer	0.12 ± 0.01	8.4 ± 1.1%	1.32 ± 0.29	1.44 ± 0.29
	|		|	|
Compound 48/80	$p < 0.01$		NS	$p < 0.01$
0.5 µg/ml,	|		|	|
10 min	2.26 ± 0.17	58.4 ± 5.7%	1.60 ± 0.21	3.86 ± 0.20

Following incubation cells were separated from incubation media by centrifugation, washed and recentrifuged; washings were added to the incubation medium. Results are averages of 5 experiments; $p <$ was determined by Student's t-test on paired samples.

Table 2. Lack of effect of aminoguanidine (AG) and compound SK + F 91488 on basal histamine and inhibition by α-fluoro methyl histidine (FMH) of increased histamine observed following compound 48/80 action on rat peritoneal fluid cells

Cell treatment[1]	Histamine (µg/flask)	Significance (vs untreated control)
Buffer	3.92 ± 0.59	
Buffer, 60 min	3.58 ± 0.32	
αFMH, 60 min	4.22 ± 1.44	$p > 0.20$
AG + SKF 91488	4.39 ± 0.72	$p > 0.20$
Buffer, 40 min; 48/80	6.10 ± 0.94	$p < 0.025$
αFMH, 20 min; 48/80	5.26 ± 1.00	$p > 0.10$
αFMH, 40 min; 48/80	3.32 ± 0.83	$p > 0.20$

[1] Except were otherwise stated, incubations were performed for 20 min at the following concentrations (µg/ml): AG, 50; compound SK F 91488, 100; αFMH, 50; compound 48/80, 0.5. Results are averages of 5 experiments.