labeled as P- or E-faces, according to Branton et al.[3].

Results and discussion. In freeze fracture, biological membranes appear as smooth surfaces (phospholipids) interrupted by globular protrusions called intramembrane particles (IMPs) (proteins)[4]. The clear identification of glial cells in freeze fracture replicas is possible beacause the IMPs profiles differ in the plasma membranes of astrocytes and oligodendrocytes[5]. The neurons present a postsynaptic membrane complex, and the dendritic membrane is readily identified as a sharply limited aggregation of 10 nm particles[6-9]. This aggregation is more conspicuous on the E-face which is generally particle-poor. The particle aggregation in the postsynaptic site of the E-face of a dendrite is shown in figure a. At high magnification, the IMPs reveal an electrondense spot, occupying the apex in some particles (fig. b). Such electrondense spots ('pores') are formed by a platinum aggregate deposited in the cavity present in the intramembrane particle during the shadowing process used in the freeze fracture technique[10]. The existence of membranous 'pores' has been hypothesized to explain the ion and hydrophile molecule diffusion occurring through the double phospholipidic layer of biological membranes[11-14].

Two types of ion movement, active and passive, can be identified across biological membranes. Active ion transport defines processes by which the ion moves 'uphill' against its electrochemical gradient by a mechanism that requires the expenditure of energy. Passive movements of ions across cellular membranes can be mediated by different types of ion channels. In same cases, these channels are large, minimally regulated and rather unselective in terms of the ions that they allow to pass. The best example of such nonselective ion channels is the nexus or gap junction[15]. These channels represent a low resistance pathway that links adjacent cells. They have recently been identified structurally[16] and are composed of units, called connexons[17], which are embedded in apposed membranes, in register and linked to each other. The connexon is a cylinder of 6 rod-shaped subunits packed to form a channel about 2 nm in diameter ('open state'). By clockwise rotation of the subunits, a 2nd structure is generated in which the subunits straighten out and slide radially, closing the channel ('closed state')[16]. It has been demonstrated that each channel of intramembrane particles in gap junction can contract gradually in response to increased concentrations of calcium. These channels are fully open at pCa 7 and closed at pCa 4.3[18]. It has recently been postulated that the plasmalemmal particles of the presynaptic terminal may be the calcium channels[14]. These channels must open in response to a change in voltage in order for the calcium current to flow. Hence each channel consists of 5 proteins each of which extends through the postsynaptic membrane. Subunits of the calcium channel have 2 possible states; in the activated state, the channel is open and when inactivated it is closed. Lliñas has postulated that in the presynaptic terminal calcium binds to a molecule called the fusion-promoting factor. In response to the binding, a certain part of the factor molecule is converted to an active state. The activated fusion-promoting factor causes synaptic vesicles to fuse with the presynaptic membrane so that they release their content of neurotransmitter. Then the activated factor returns to an inactive state. Meanwhile the neurotransmitter molecules are opening channels in the postsynaptic membrane so that ionic current flows and membranes become depolarized[14]. These hypothetical postsynaptic channels may be associated with the postsynaptic membrane particles (transmitter-dependent channels)[14].

On the basis of the structural similarity between the cavity particles of gap junctions[16,17,19] and isolated particles of different cells[10] and those observed in the dendrite postsynaptic membrane of rat neonatal cerebellar cortex, it is postulated that the latter are provided with a channel linking the postsynaptic cytoplasm with the synaptic cleft; however, its significance remains to be clarified.

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Acetylcholinesterase localization at synapses in chick embryo ciliary ganglion

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Summary. The cytochemical localization of acetylcholinesterase (AChE) at calyciform synapses of the chick ciliary ganglion during embryonic development has been investigated. AChE activity is present at the surface membrane of newly formed calyciform synapses and closely follows the progressive enlargement of the synaptic area. The occurrence of a retrograde iris-dependent influence on ganglionic AChE is considered. AChE seems to be a suitable marker for synaptic maturation.

It is known that cholinergic activities undergo characteristic changes during embryonic life in the chick ciliary ganglion (CG)[1], an autonomic parasympathetic ganglion with a typical cholinergic transmission[2]. The calyciform nerve ending is a giant presynaptic terminal which surrounds large surface areas of the ciliary neurons, one of the 2 cell

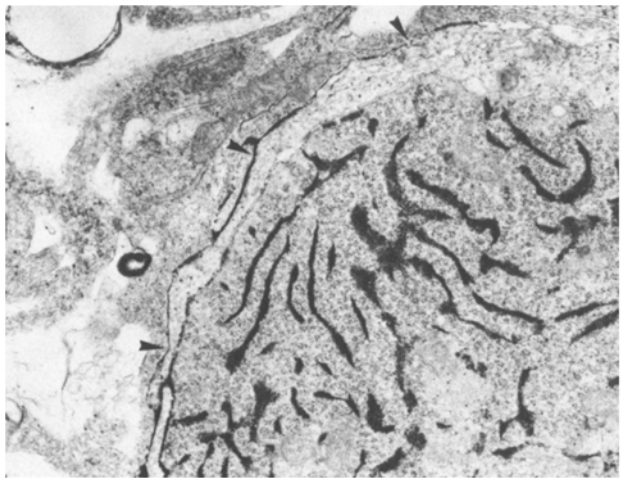

Figure 1. 9-day chick embryo CG. An early calyciform synapse is partially and unevenly marked by AChE reaction (arrows). Note the occurrence of end-product within the cisternae of the rough endoplasmic reticulum. ×21,000.

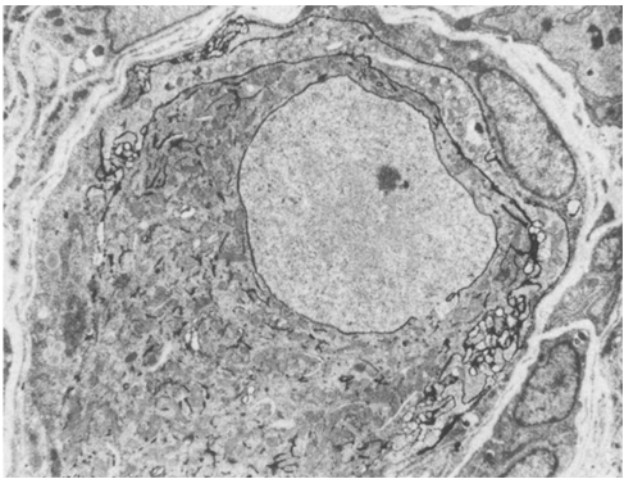

Figure 3. 15-day chick embryo CG. An AChE-labelled calyciform synapse surrounds a great surface area of a ciliary neuron. AChE reaction intensely and evenly labels the calyciform synapse. ×5,800.

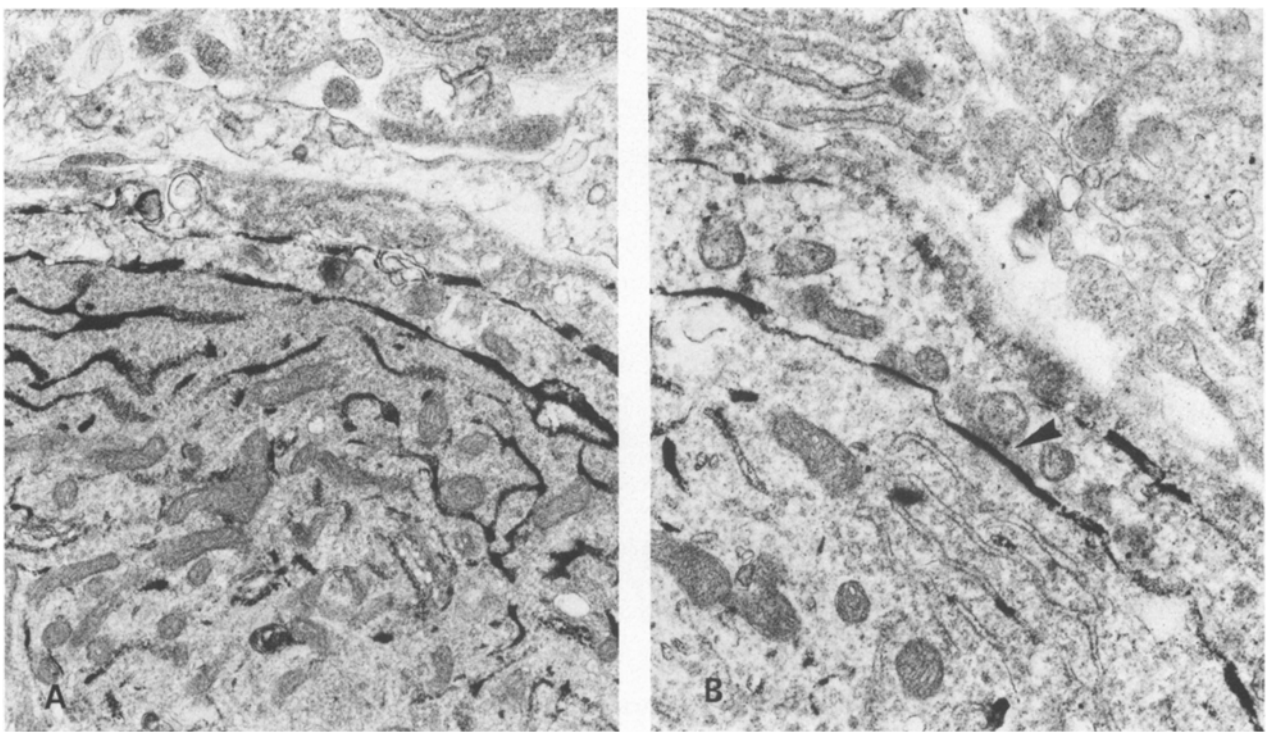

Figure 2. 12-day chick embryo CG. *A* The calyx surface membrane appears heavily labelled by AChE reaction, although in a discontinuous way. Note the more marked labelling at the contact with the neuronal surface. ×21,150. *B* An 'active site' of a calyciform synapse is intensely labelled by the reaction product (arrow). ×27,000.

populations typical of the CG[3-5]. The present report deals with the ontogenetic development of AChE distribution at the calyciform synapse during embryonic maturation.

Materials and methods. Ciliary ganglia from 9-, 12- and 15-day White Leghorn chick embryos were carefully dissected out, fixed for 2–3 h in a mixture of aldehydes (paraformaldehyde 4% plus glutaraldehyde 2.5%) in cacodylate buffer pH 7.3 and washed overnight with the same buffer. For ultrastructural localization of AChE the ganglia were preincubated in a medium without substrate for 20 min and then incubated for a further 45 min at 4 °C according to Lewis and Shute[6], using acetylthiocholine iodide as a substrate. The specimens were then postfixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in epon. Thin sections were finally examined in a Philips EM 300 electron microscope.

Control incubations were performed using the following inhibitors: BW284C51 (1,5-bis-4-allyldimethylammoniumphenylpentan-3-one dibromide) 5×10^{-5} M, an inhibitor of AChE, eserine 10^{-4} M, an inhibitor of cholinesterases in general and iso-OMPA (tetraisopropyl pyrophosphortetramide) 2×10^{-5} M, an inhibitor of pseudocholinesterase. Finally, control ganglia were incubated in a medium lacking acetylthiocholine iodide.

Results. As soon as the calyx first appeared, i.e. at 9 days of incubation (d.i.), a slight and discontinuous reaction product was found along the surface membrane of this nerve terminal (fig. 1). Furthermore, as expected, endocellular AChE was observed in the cisternae of the rough endoplasmic reticulum, as proviously described[7]. At 12 d.i. the surface membrane was much more heavily labeled than at 9 d.i., although in a segment-like way (fig. 2, A). The intensity of labeling was quite variable and generally more marked at the 'neuronal' side of the calyx surface membrane than on the 'satellite' side, i.e. the side facing the satellite cell (fig. 2, A).

Quite often 'active sites' - which occur at points along the calyx itself - were markedly labelled as compared to adjacent membrane areas (fig. 2, B). At 15 d.i. - when the calyx is known to reach full maturation[8] - AChE reaction was intense and uniform, clearly labeling large surface areas typical of such nerve terminals at this developmental stage (fig. 3).

Control studies performed with BW284C51, an inhibitor of AChE, demonstrated no cytochemical reaction, indicating that the reaction product obtained in our experiments is indeed due to AChE activity. Finally under our experimental conditions we failed to observe diffusion of the reaction product.

Discussion. AChE activity localized at the surface of the ciliary neurons was first observed at 9 d.i., i.e. at the time of the first appearance of the calyciform synapse, the end product labeling both the neuronal and the satellite sides of the calyx surface membrane. Such a pattern of staining is not surprising, since AChE is known to occur all along the outer surface of the axonal membrane in cholinergic nerves[9]. During ontogenesis, however, differences emerge in AChE distribution between the neuronal side of the calyx and the satellite side, possibly due to a selective redistribution of AChE following the establishment of the axosomatic contact. Moreover, the fact that at 12 d.i. the neuronal side of the calyx appears more heavily labeled than the satellite one is in line with previous findings indicating that AChE is released from nerve terminals into the synaptic cleft, possibly serving as one source of postganglionic AChE[10]. In this context it must be emphasized that a heavier labeling of the neuronal side as compared with the satellite one still occurs in the adult CG[11]. From 12 to 15 d.i. the reaction product increases concomitantly with the ontogenetic enlargement of the synaptic area. There are in addition active sites which at these developmental stages appear to be markedly labeled by AChE reaction and are conceivably involved in ganglionic transmission. In this connection, however, it must be recalled that in the chick CG neurotransmission is fully developed at 7 d.i.[2] in spite of a minimal morphological differentiation of synapses at that developmental stage. Thus it is likely that AChE occurring in the newly formed calyx (9 d.i.) may already be involved in synaptic transmission.

The calyx-related distribution pattern of AChE on ciliary neurons appears to be closely linked to the establishment of the calyciform synapse, the calyx possibly acting as an inducing factor for a selective redistribution of AChE. During this period, i.e. from 9 to 15 d.i., functional synapses are forming between the ganglion and its target, i.e. the iris[12], so that retrograde iris-dependent influences on ganglionic AChE are already at work[1,13].

The overall distribution of ganglionic AChE is definitively stabilized only after the final pattern of neural connections has been established.

In conclusion AChE appears to be a suitable marker for synaptic maturation, and may also be useful for studying neuron-target cell interactions in the developing nervous system.

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Effect of ouabain on volume regulation of rabbit kidney cortex slices in hypo-osmotic media[1]

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Summary. The volume regulation process at work in rabbit kidney cortex slices submitted to hypo-osmotic media show both a swelling limitation and a volume readjustment phase. Swelling limitation is Na^+ dependent and is blocked by ouabain 10^{-3} M. There is, however, no need to implicate the activity of a ouabain sensitive Na^+/K^+ pump in this process.

It is now generally recognized that animal cells can regulate, at least partly, their volumes when subjected to hypo-osmotic conditions. During the past decade, this process has been shown to occur in a variety of tissues and cell lines from invertebrates and vertebrates (for recent reviews[2-10]).

Volume regulation is, in most cases, associated with a loss of intracellular K^+ and, especially in aquatic invertebrates, with a decrease in the level of free amino acids. In mammalian kidney, volume control appears to be essentially associated with a decrease in intracellular Na^+ [11-14].

Further, it has been shown that ouabain inhibits volume regulation in different kidney preparations[10,11]. This has been taken as evidence to state that the Na^+/K^+ ouabain sensitive pump is implicated in volume control in this tissue. This is at variance with what is found in most other tissues; the lack of effect of ouabain on volume readjustment has been reported many times[3,5,9]. In an attempt to clarify these problems, we have undertaken a study of volume regulation in rabbit kidney slices and of the effects of ouabain on this process.