Cholesterol and Sterol-Like Compound in Developing Chick Embryo Sensory Ganglia

In a previous paper[1] we reported on the isolation of a compound with a still unknown steroid structure, which is present in 8-day-old chick embryo sensory ganglia, accounting for about 5% of the dry weight. This relatively high percentage favoured the hypothesis that this compound might be a cell membrane constituent in this early stage of development. This compound was also found to be present in other tissues but at a much lower concentration[2].

In the present work, the quantitative determination of cholesterol and of our sterol-like compound, related to the protein content of developing sensory ganglia from chick embryos from 6–14 days, will be described. The relationship between these 2 steroids, and their participation in the myelinization process is discussed.

Methods. Sensory ganglia explanted from 6- to 14-day-old chick embryos were homogenized in an aqueous NaCl-solution (0.9:100, w/v).

An aliquot of the homogenate, 90%, was extracted with chloroform-methanol (2:1, v/v) for the extraction of total lipids according to FOLCH, LEES and SLOANE-STANLEY[3]; 2 extractions were carried out with 5 ml of the mixture per 100 homogenized ganglia. The chloroform extract was dried over anhydrous sodium sulphate and then concentrated in vacuo. The total lipid extract was saponified in a 2*N*-KOH solution in aqueous 50% (v/v) methanol, using 25 ml/100 ganglia, for 24 h at room temperature in darkness. To the unsaponifiable material a known quantity of stigmasterol was added and then the mixture was separated on a Perkin-Elmer 800 gas-liquid chromatograph provided with a column 1.80 m long by 3.0 mm internal diameter packed with Chromosorb G (80–100 mesh) impregnated with SE-30 (2%). Injector and column were made of glass; the injection temperature was 270 °C; the flow of nitrogen gas was 40 cm³/min. The column temperature was programmed from 125 up to 275 °C with a constant temperature increase of 8.33 °C/min. The peak areas of our sterol-like compound and of cholesterol were referred to the area of stigmasterol for the quantitative measurements.

The remainder (10%) of the homogenate was used for the determination of the total protein content according to the method of LOWRY[4], using bovine serum albumin as reference.

Results and discussion. A graphic plot of the total amount of protein per sensory ganglion (Figure 1) in 6- to 14-day-old chick embryos represents a *S*-curve, which is generally found in growth processes, increasing from 2–11 μg protein.

The amount of cholesterol/ganglion (Table) increases from about 0.02 μg at 6 days to 0.60 μg at 14 days, while, however, the sterol-like compound has an increment from about 0.10–0.25 μg, which value is reached already after 8 days of development and then remains at the same level. This implies that the ratio sterol-like compound versus protein (Figure 2) decreases and that cholesterol versus protein shows a curve similar to that of protein increment in developing ganglia. This difference between the 2

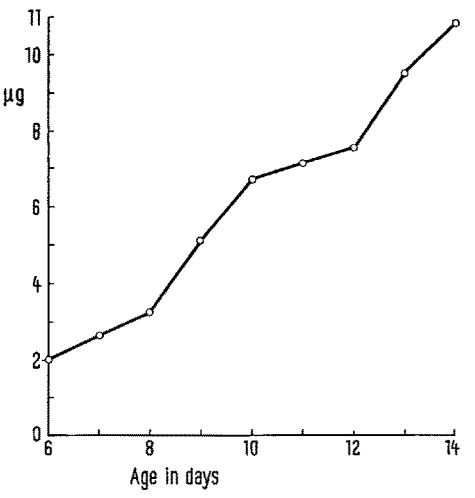

Fig. 1. Protein content of 6- to 14-day-old chick embryo sensory ganglia, determined according to the method of LOWRY, expressed in μg/ganglion. Each value is the medium of 10 different measurements.

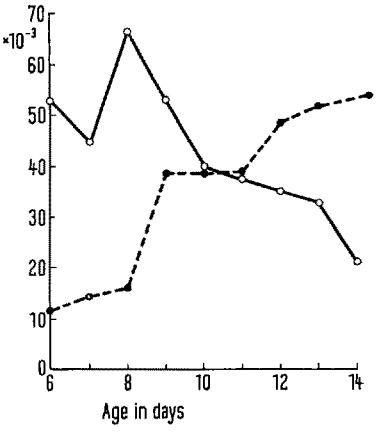

Fig. 2. Amount of sterol-like compound versus total protein (o——o) and cholesterol versus total protein (•---•), expressed in μg per ganglion. The quantities of sterol-like compound and of cholesterol were determined by separation of the unsaponifiable lipid material on a gas-chromatographic column, using stigmasterol as internal standard. Total protein was measured according to the method of LOWRY. The results are the medium of 5 different experiments.

Amount of sterol-like compound and of cholesterol in sensory ganglia of 6- to 14-day-old chick embryos

Days	Sterol-like compound (μg)/ganglion	Cholesterol (μg)/ganglion
6	0.106 ± 0.008	0.023 ± 0.002
7	0.116 ± 0.010	0.041 ± 0.003
8	0.217 ± 0.004	0.053 ± 0.004
9	0.268 ± 0.009	0.198 ± 0.007
10	0.266 ± 0.011	0.256 ± 0.009
11	0.269 ± 0.014	0.274 ± 0.007
12	0.263 ± 0.011	0.364 ± 0.021
13	0.311 ± 0.016	0.489 ± 0.030
14	0.232 ± 0.007	0.580 ± 0.019

The results are the mean of 5 measurements (± S.E.M.).

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steroids is the interesting feature of the ganglia. The cholesterol content during development of the sensory ganglia shows 2 important steps (Figure 2): one after 8 days and a less pronounced increase after 11 days. This change in the synthesis is most probably due to the myelinization process, as has also been shown by MEDDA and BOSE[5], who reported that in the spinal cord of chick embryos there was an increase of cholesterol formation up to 8 days of incubation. Comparing the increase of cholesterol per ganglion with the increase of protein/ganglion, one observes that cholesterol increases 30 times and protein only 5 times. This observation agrees with the statement of FOLCH-PI[6] that during the myelinization process the protein content decreases relatively compared with lipid synthesis. Further it is interesting to note that the sum of cholesterol and sterol-like compound per protein remains almost constant at approximately 85×10^{-3} during the whole period from 6 until 14 days. It may be possible that the sterol-like compound is a biological precursor of cholesterol, which ultimately takes the place of the unknown compound after a certain stage of cellular development has been reached. Already FUMAGALLI and PAOLETTI[7] have observed that during embryonic growth there is a change in the steroid pattern. However, chemically the sterol-like compound does not seem to be a precursor of cholesterol because this compound has an absorption maximum in the UV-region at 286 nm in ethanol indicating a relative high state of insaturation, and it is known in steroid biosynthesis that all the dehydrogenation steps occur after the formation of cholesterol. Furthermore, orientative mass spectrometric measurements have indicated a molecular weight of the sterol-like compound which coincides with a C_{21}-steroid.

In the case that the sterol-like compound is not a biochemical precursor of cholesterol, it is difficult to envisage its role in the myelinization process. To this observation one can add the results obtained during previous work[1] showing that a specific nerve growth factor (NGF)[8] has an inhibitory effect in vitro on the synthesis of this sterol-like compound in 8-day-old chick embryo sensory ganglia, reducing its quantity of about 25% in a 4 h incubation period.

The coincidence between the period, in which the nerve cells are responsive to NGF, which elicits nerve fibre outgrowth between the sixth and tenth day of development and the maximum concentration of the sterol-like compound in the ganglia between the eighth and ninth day may suggest a possible relationship between the action of NGF and the disappearance of the sterol-like compound in the ganglia[9].

Riassunto. Sono stati misurati per via gascromatografica i livelli di colesterolo e di un composto steroidico a struttura finora sconosciuta nei gangli sensitivi di embrione di pollo di 6 fino a 14 giorni di età. I risultati ottenuti mostrano che durante lo sviluppo lo steroide a struttura sconosciuta ha una massima concentrazione a 8–9 giorni per poi decrescere fino a 14 giorni, mentre il colesterolo presenta una tipica curva di accrescimento ad *S* con due punti di flesso, uno a 8 ed uno a 11 giorni.

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β-Fructofuranosidase Activity in Pea Seeds

Dormant seeds of Leguminosae contain a large amount of reserve oligosaccharides of the raffinose family which are hydrolyzed in the first phases of germination. Enzymes responsible for their splitting, β-fructofuranosidase (β-D-fructofuranoside – fructohydrolase 3.2.1.26)[1–3] and α-galactosidase, were found in dormant seeds and their activities increased during germination. In *Phaseolus vulgaris*, 2 types of β-fructofuranosidase were found[1,2]: in dormant seeds, alkaline β-fructofuranosidase with a pH optimum at 7.7 was present only, while during germination the activity of acid β-fructofuranosidase with pH optimum at 5.0 increased. In dormant *Vicia faba* seeds the alkaline β-fructofuranosidase was found too, but the substrate specificity was different[3]. β-Fructofuranosidase from *P. vulgaris* seeds was specific to sucrose. Substrate specificity of *V. faba* enzyme was similar to the specificity of β-fructofuranosidase of other sources. Transferase activity of β-fructofuranosidase was observed in the case of acid β-fructofuranosidase from seedlings of *P. vulgaris*[2] and in the case of alkaline β-fructofuranosidase of *V. faba* seeds[3]. Alkaline β-fructofuranosidase from dormant *P. vulgaris* seeds did not exhibit transferase activity[2].

In the course of the study on the metabolism of saccharides in pea seedlings, the nature of enzyme hydrolyzing sucrose present in dormant seeds and seedlings was investigated. In dormant pea seeds, only the alkaline β-fructofuranosidase activity was determined; in 10-day pea seedlings both types of β-fructofuranosidase activity – alkaline and acid – were found. Properties of alkaline β-fructofuranosidase were studied in detail.

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