cantly. An unidentified peak, travelling between leucine and tyrosine in bile, was not found in any serum sample.

Discussion. While there have been many studies of the protein content of bile[5], very little information is available about the amino acid composition of bile. Miller[6] analysed cadaveric bile and found free lysine, tyrosine and glycine which he considered represented the excretion of waste products. Dassi and Gianni[7] examined human bile obtained during laparotomy and detected water-soluble substances reacting with ninhydrin, which they considered compatible with the presence of free amino acids or polypeptides. The present study is therefore the first to quantify the amino acids in bile and to compare the pattern of amino acids in bile with those found in serum.

The high concentrations of acidic and sulphur-containing amino acids in bile and the relative lack of basic amino acids has not been explained. In part, the concentrations in bile may reflect the concentrations of the amino acids inside the hepatic cells, since it has been shown that there are significantly greater amounts of some amino acids (glutamate, aspartate and glycine) in the liver cells than in serum[8]. However, other amino acids are also found to be concentrated in the hepatocytes (e.g. alanine) but are not found concentrated in bile, while amino acids which are not concentrated by the liver cells (e.g. cystine) may be found in greater amounts in bile than in serum. Lysine has been shown to be specifically transported by the mucosa of the gall bladder, but so are methionine and glycine[9], so that the relative lack of lysine in the bile is probably not attributable to selective transport of the lysine out of the lumen of the biliary tract.

Unlike proteins, which appear in bile by processes involving bulk transfer and molecular sieving of the plasma proteins[10,11], it appears that the transfer of amino acids into the bile involves specific transport processes. It has previously been shown that organic anions[12] and cations[13] can be secreted into bile by active transport processes but multiple excretory processes are probably involved[14] and the role of these processes in the transport of amino acids has not been studied. In the small intestine, the transport of the neutral, acidic and basic amino acids is highly specific[15] so that there may be similar processes involved in the hepatic secretion of these amino acids, in view of the significantly different handling of these 3 groups of amino acids by the liver cells.

The function of the biliary amino acids has not been defined, but amino acids are known to stimulate pancreatic exocrine secretion[16] and, perhaps in conjunction with bile salts (which also stimulate pancreatic secretion[17]) may have some functional role in the early stages of the digestive response to the entry of food into the alimentary tract.

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Nerve endings isolated from chick embryonic optic tectum. 2. Developmental aspects of synaptosomal membrane

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Summary. Fractions enriched in nerve endings (synaptosomes) have been isolated from chick embryonic optic tectum during development. After osmotic shock, these fractions appeared to be enriched in membranes which during development acquire typical features of mature synaptosomal membranes.

The development of improved fractionation techniques has permitted biochemical and morphological studies to be carried out into the nature of individual components of nerve endings (synaptosomes).

The most attention has been focused on synaptosomal membranes, both in analysis of gross composition and identification of specific components[1] both in ultrastructural organization of synaptic densities[2], in correlation with its critical role played in synaptic transmission. It is also generally accepted that synaptosomal membrane is to a certain extent involved in neuronal receptor recognition and specificity of cell adhesion during the development of neuronal circuits[3]. Consequently, isolation of embryonic synaptic membrane can be regarded as an essential step for biochemical and morphological studies on properties of maturing synaptic complexes.

	16th		18th		2nd	
	a)	b)	a)	b)	a)	b)
Homogenate	100	48.35	100	91.45	100	66.00
Crude mitochondrial fraction	19.86	9.60	17.32	15.84	20.72	13.68
Synaptosomes	4.34	2.10	6.90	6.31	5.21	3.44
Membranes	1.86	0.90	5.68	5.20	7.15	4.72

a) Percentage of protein content of each fraction based on the homogenate being 100%; b) total protein content of the fractions (mg).

In the preceding paper[4], we discussed some morphological aspects of nerve endings isolated from developing optic tectum of chick embryo. We observed a progressive 'maturation' of synaptosomes which showed an increase in number of vesicles and synaptic thickenings. In this paper, we describe fractions enriched in synaptosomal membranes obtained after osmotic shock of those synaptosomal fractions.

Materials and methods. Synaptosomal fractions have been obtained as described in preceding work. Synaptosomal band B of the gradient was removed, diluted with 4–5 volumes of 0.32 M sucrose and pelletted at 80,000 ×g for 30 min. The synaptosomal pellet was resuspended in a large volume of 6 mM Tris (pH = 8.5) and osmotically shocked for 90 min. The shock was made at pH 8.5, since at neutral or slightly acid pH, mitochondria do not effectively resolve from membranes[5]. After shock, synaptosomal membranes fraction was pelletted with centrifugation at 20,000 ×g for 30 min.

Small portions of fractions were fixed for 30 min in ice-cold 2.5% glutaraldehyde in 0.1 M phosphate buffer and

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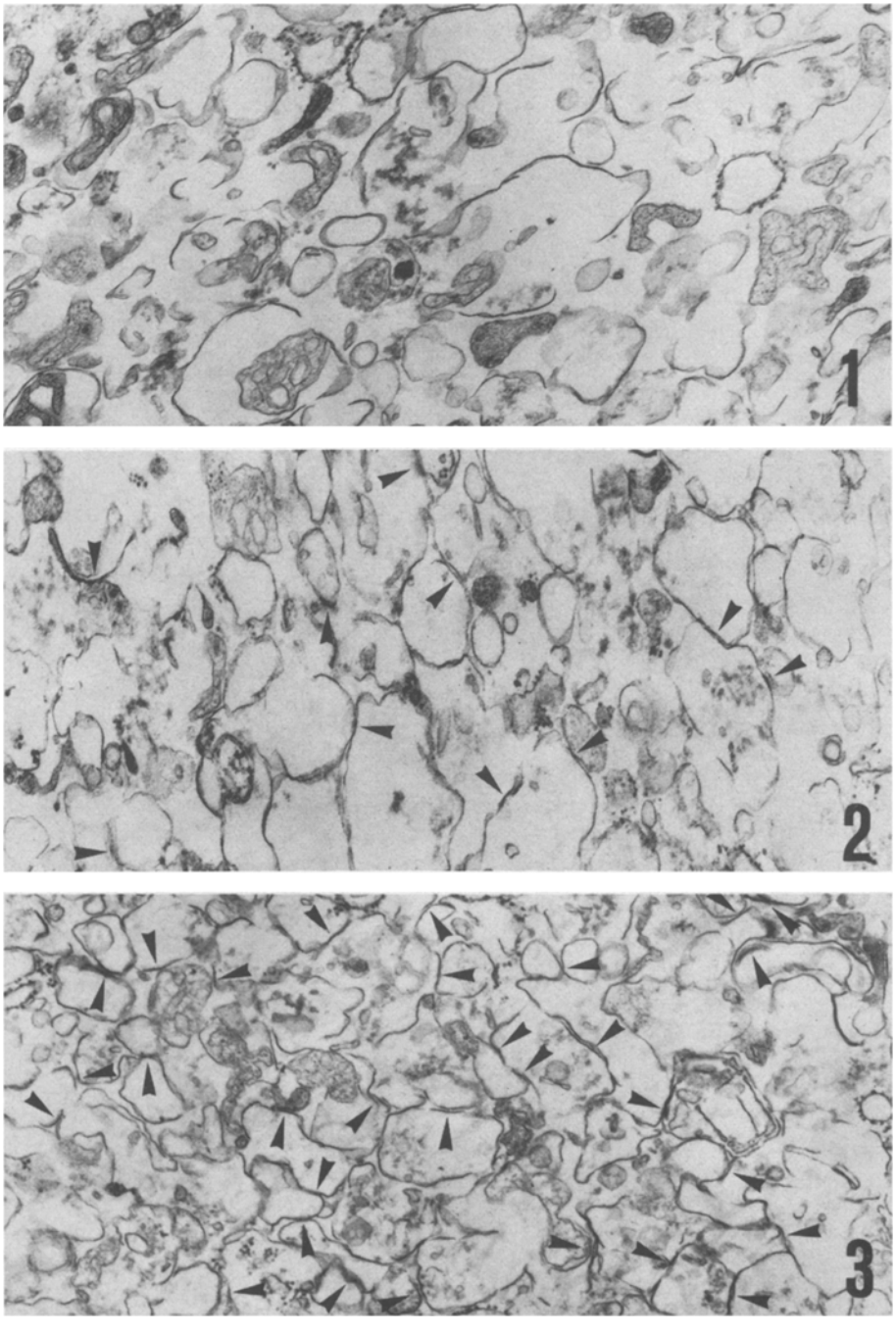

Electron micrographs showing fractions obtained after osmotic shock on nerve endings isolated from chick embryonic optic tectum. ×30,400.

Fig. 1. 16 days old embryos. Fraction is enriched in membrane profiles without thickenings or synaptic densities recognizible. Fig. 2. 18 days old embryos. The figure shows a major amount of round membrane profiles, many of which show attached synaptic densities (arrows). Fig. 3. 2 days old chicks. Fraction appears full of membrane profiles about the size of synaptosomes with very recognizible pre- and postsynaptic thickenings (arrows).

spun in order to obtain pellicles less than 500 μm thick. After postfixation in situ with 1% buffered OsO_4, pellicles were embedded in a Epon-Araldite mixture. Sections were cut at right angles to the surface of pellicles through the whole pellet. Then they were stained and observed on a Siemens Elmiskop I A electron microscope. Proteins were measured according to Lowry et al.[6].

Results. At 16th day of incubation, the fraction obtained with osmotic shock from synaptosomal fraction appeared relatively poor in membranes, which were to some extent disrupted and almost devoid of synaptic thickenings (see figure 1). Microsomes, free mitochondria and a few membrane profiles containing residual synaptic vesicles adhering inside could be also observed.

At 18th day of incubation, the fraction appeared enriched in membrane profiles about the size of synaptosomes, many of which have attached synaptic thickenings. Free mitochondria and microsomes content was lower than in younger embryos (see figure 2). In chicks, the fraction appeared full of well-maintained membranes with very recognisable symmetrical and asymmetrical synaptic thickenings, whereas contaminating structures appeared sharply diminished (see figure 3).

Relative protein content of membrane fraction of 16 days old chicks was about 30% of 18 days old embryos value and about 25% of chicks value. In contrast, the difference in relative as well as in absolute content of proteins between synaptosomal fraction before and after osmotic shock appeared to decrease during maturation.

Discussion. Our morphological observations on these fractions enriched in membranes seem to be perfectly in agreement with data we obtained on synaptosomes isolated from chick embryonic optic tectum (see preceding paper)[4]. We observed a progressive increase in maturation of synaptosomes with augmentation of synaptic thickenings and a decrease of contamination of the synaptosomal fraction parallel to the progressive development of tectal synaptogenesis. Synaptosomal membranes isolated in older stages appeared also quite similar to that isolated from adult rat brain[5], and from 6–8 days old chicks[7]. Consequently, it is highly probable that membranes obtained after osmotic shock of synaptosomal fractions are largely synaptosomal and synaptic membranes which show a progressive maturation. However, no data are so for available about morphology of embryonic synaptosomal membranes. In addition, from a morphological point of view, it is not possible quantitatively to measure contamination by microsomal membranes and other subcellular elements which may appear during preparation of membrane fraction by centrifugation, even if a relative low speed has been used. Consequently, further biochemical and enzymatic studies are necessary for characterization of these membrane fractions. With this reservation, nevertheless, we think that this method can be used as a first step to obtain synaptosomal and synaptic membranes at relatively precocious stages of development, and it might provide an improved technique to prepare embryonic synaptosomal membranes from shocked mitochondrial fraction.

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Electro-mechanical noise in atrial muscle fibres of the carp[1]

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Summary. Steady membrane voltage fluctuations have been observed in atrial muscle fibres of the carp. These voltage fluctuations produce minute mechanical oscillations, as revealed by an interference contrast microscope. The steady voltage fluctuations may be related to abnormal automaticity in the heart.

Fluctuation phenomena have revealed useful information on molecular mechanisms operating in excitable membranes[2–6]. We now report the finding of steady membrane voltage fluctuations which produce minute mechanical oscillations in atrial muscle fibres of the carp. The voltage fluctuations can grow and lead to repeated spike activity, and may thus be linked to abnormal automaticity in the heart.

Materials and methods. Experiments were performed on isolated carp (Cyprinus carpio) atria, from which the pacemaker regions have been removed. Muscle fibres in the carp atrium are relatively large (up to 25 μm in diameter) and stable intracellular recording can be achieved for at least a few minutes. The atria were continuously perfused with carp Ringer of the following composition[7]: (mM) NaCl 120, KCl 2.7, $CaCl_2$ 2.9, $MgCl_2$ 1.0, $NaHCO_3$ 10, pH (7.8–8.0). The intracellular potential was recorded with glass micropipettes having a resistance of 15–25 MΩ when filled with 3 M K-citrate or KCl. Signals were recorded on paper chart (Grass Model 79) or on magnetic tape (Hewlett Packard 3955 System) and analysed by a digital computer (Varian 620L-100).

Results and discussion. Figure 1 shows a typical recording of voltage fluctuations in atrial muscle cells. Immediately after penetration, a DC potential of –79 mV was recorded, and there was a large increase in the voltage fluctuations. The average RMS value ± 1 SEM of the voltage fluctuations was 66 ± 11 μV (n = 14). Spectral analysis of the noise showed most of the power to be concentrated at frequencies below 3 Hz with the addition of a hump at

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