37 °C, each group of embryos is dehydrated and fixed by transferring them to a depression in another spot test plate containing a mixture of cold, 100% methanol and glacial acetic acid (3:1). The embryos are allowed to remain in the solution for 10 min. During this treatment the test plates are kept on ice. Attempting to handle more than 10 embryos through the hypotonic treatment and fixation results in a loss of material.

In order to disassociate the cells of the fixed embryos, each blastocyst is next placed onto an acid cleaned glass slide and one drop of a solution containing EDTA, deionized water and glacial acetic acid is applied directly to the embryo. This solution is made by first dissolving 0.01 g EDTA in 50 ml deionized water. On the day of use, an aliquot of this solution is diluted with deionized water (1:1). Glacial acetic acid is then added to the diluted solution (3:1). As soon as the cells of the blastocyst begin to disassociate from each other, three consecutively applied drops of cold, 100% methanol:deionized water; glacial acetic acid (9:4:3) are placed directly onto the embryo causing the cells to complete disassociation and to lyse. The liquid is allowed to evaporate between applications to the extent that the embryonic cells become visible by microscopic examination. The rapid spreading of the drops of this hypotonic solution is critical for good chromosome spreads. If the drops do not spread out over the slide quickly, the chromosomes remain clumped and are not distinguishable.

Immediately following the partial evaporation of the third drop of the hypotonic solution, three drops of 100% methanol:glacial acetic acid (3:1) are applied, allowing each to evaporate to the extent described in the previous step. The application of this solution dehydrates the material and increases its adhesiveness to the slide. The slide is then placed on a warming plate (37 °C) and allowed to dry. The disassociated and lysed cells are stained with giemsa (Gurr; Hopkin and Williams, Co. Essex, England). Optimum results are obtained if all solutions are prepared immediately prior to use.

Using the described technique we are able to analyze > 70% of the embryos prepared for karyotypic studies. The figure is typical of the karyotypes obtained by this method.

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Incorporation of ^{32}P into renal phospholipids of mice during postnatal growth

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Summary During the first 40 days of life the rate of incorporation of ^{32}P into total phospholipids and into phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin, diphosphatidylglycerol, phosphatidic acid and phosphatidylinositol of mouse kidney was by some 25–35% higher than in older animals. Results suggest a different involvement of cellular membranes during of normal and compensatory renal growth.
Key words. Mice; phospholipids; kidney growth.

Cellular membrane synthesis occurs during postnatal growth of the kidney. Rapidly growing neonatal rat kidneys contained high levels of choline kinase activity and the level gradually declined during the first month of life[1]. During the early phase of compensatory renal growth incorporation of ^{32}P into renal phospholipids, particularly phosphatidic acid and phosphatidylinositol, was increased[2]. In this article we describe investigations, using carrier-free ^{32}P, of phospholipid metabolism during postnatal growth of the kidney, in order to compare the data (on incorporation of ^{32}P into phospholipid fractions) for normal kidney growth with those obtained previously during compensatory kidney growth[2].

Materials and methods. C57BL/GoZgb mice, aged 1–120 days, were used in this study. The mice that were 20 days old or older were all males. The 1-day-old puppies were used within 24 h of their normal vaginal birth. The 1-, 5- and 10-day-old mice were used not later than 15 min after separation from the dams. The young ones suckled until 20 days after parturition. The non-suckling animals (over 20 days old) were maintained on a standard laboratory diet (Sljeme, Zagreb, Yugoslavia) and had free access to tap water.

2 h before sacrifice all mice of different ages were injected i.p. with 37 kBq ^{32}P per g body mass as carrier-free ^{32}P-labeled sodium orthophosphate (purchased from Vinča, Beograd, Yugoslavia).

The animals were sacrificed by decapitation. The kidneys of suckling mice were not purposely decapsulated, but capsules were often damaged during dissection. The kidneys of older mice were decapsulated by slitting the capsule at one end and squeezing gently between index finger and thumb. Each kidney was washed with isotonic saline, blotted on filter paper and weighed on a torison balance. The kidneys were pooled from six 1-day-old mice, four 5-day-old mice and three 10-day-old mice. From 20-day-old mice both kidneys were used and in the older mice all estimations were performed on right kidneys only.

Tissue samples were homogenized in 2 ml of ice-cold 0.3 N perchloric acid with a Potter-Elvehjem glass homogenizer as described previously[2]. The phospholipids were extracted by the method of Folch et al.[3] and were analyzed by thin layer chromatography. Aliquots were placed on two silica gel 60 G 254 plates (E. Merck AG, Darmstadt, Federal Republic of Germany), one plate was run in chloroform/methanol/acetic acid/water (65:50:1:4) and the other in chloroform/methanol/saturated ammonia/water (65:35:2:3). Phospholipids were visualized using iodine vapor or UV light. R_f values were compared with those of known standards purchased from Sigma Chemical Co,

St Louis, Mo., USA. Phospholipids were scraped into scintillation vials containing 10 ml of a scintillation cocktail containing: 5 g 2,5-diphenyloxazole and 50 mg 1,4-bis[2-(5-phenyloxazolyl)] benzene in 1 l toluene, and were counted in a Beckman LS-250 scintillation counter. Recovery of the radioactivity from each fraction of phospholipids was 80–85%. Phospholipids were measured by phosphate determination following the method of Bartlett[4].

All results were expressed as the mean ± SEM. The result for 1-day-old mice was obtained from a single pool of kidneys. The significance of the difference between means of each group at different ages was evaluated by the t-test for independent data[5].

Results and discussion. The results of measurements of the wet mass of right kidney during postnatal growth (fig. 1A) are in general agreement with those obtained by Priestly and Malt[6] on Ham/ICR albino mice.

Kidney phospholipid concentration (fig. 1B) significantly increased ($p < 0.01$) between the 30th and 40th day of age. A similar observation was made by Pushpendran and Eapen[7] during postnatal development of kidneys of male Swiss mice. However, in their study there was no change in kidney phospholipid concentration of female mice of the same age, indicating that the increase of kidney phospholipid concentration could be caused by puberty in the male mice.

The rate of incorporation of ^{32}P into total phospholipids and into seven investigated fractions (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin, diphosphatidylglycerol, phosphatidic acid and phosphatidylinositol) in the kidney during postnatal growth showed an identical pattern (fig. 2). During the first 40 days of life the rate of incorporation of ^{32}P into each type of renal phospholipid was higher (at the level of at least $p < 0.02$) by some 25–35% than in the older animals. This increased incorporation of ^{32}P into phospholipids is unlikely to be caused by an increased uptake of ^{32}P into cells, as estimated by the acid-soluble pool, because the acid-soluble pool did not change during postnatal growth of the kidney (fig. 1C).

The same level of increased ^{32}P incorporation into phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin and diphosphatidylglycerol during the rapid phase of normal kidney growth described in this article, and during compensatory renal growth observed earlier[2], together with increased levels of choline kinase activity[1], show that cellular membrane synthesis occurs during both normal and compensatory renal growth.

Phosphatidic acid and phosphatidylinositol are components of the phosphatidylinositol turnover cycle. Stimulation of this cycle has been suggested as an important event in a wide range of

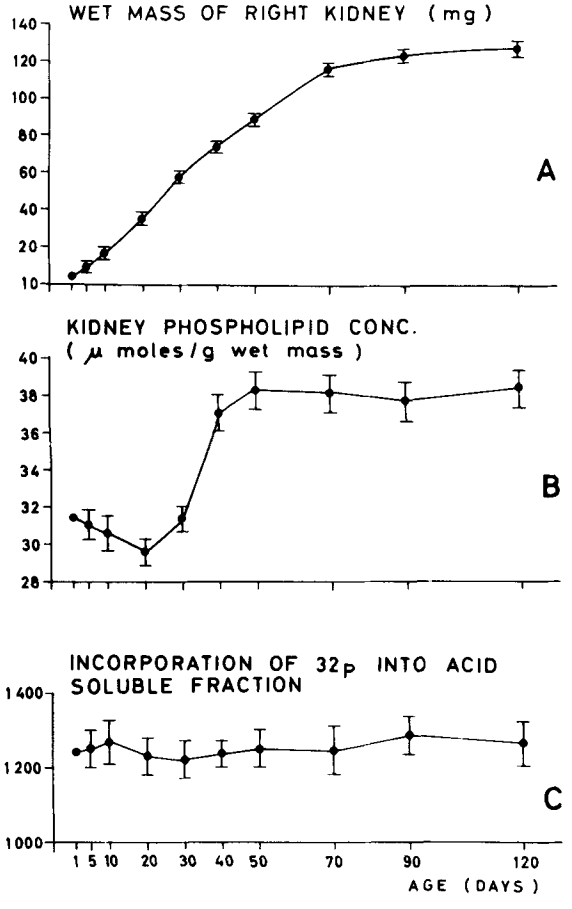

Figure 1. Wet mass of right kidney (A), kidney phospholipid concentration (B) and incorporation of ^{32}P into acid-soluble fraction of kidney (C) during postnatal growth. The incorporation of ^{32}P into acid-soluble fraction was expressed as (cpm per g kidney mass/cpm injected per g body mass) × 10^3. Each point represents the mean of at least three tissue samples as described in 'materials and methods' section and the vertical bars represents 2 SEM.

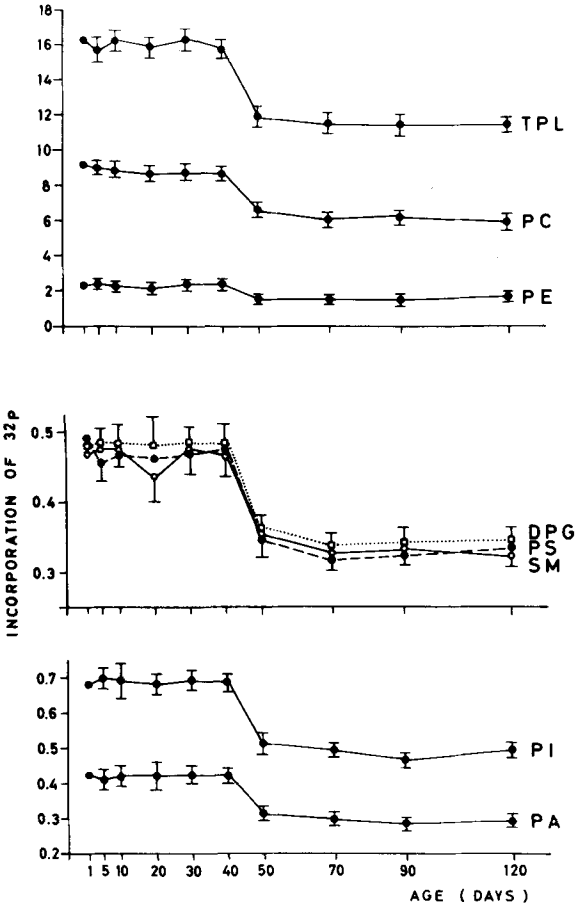

Figure 2. Incorporation of ^{32}P into total phospholipids (TPL), phosphatidylcholine (PC), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylserine (PS), sphingomyelin (SM), phosphatidylinositol (PI) and phosphatidic acid (PA) of kidney during postnatal growth. For each fraction the incorporation was expressed as cpm in investigated fraction per μmole total phospholipid phosphorus/cpm injected per g body mass. The number of animals used, and significance of the error bars are as in figure 1.

activated cells, particularly during initation of cell growth and proliferation[8,9]. Our previous results showed that during compensatory renal growth stimulation of inositide metabolism occurs[2]. During postnatal renal growth the incorporation rate of ^{32}P into phosphatidic acid and phosphatidylinositol showed an identical pattern with other phospholipids, and did not show a phosphatidylinositol effect (fig. 2). These findings are in accord with the hypothesis that there must be a significant difference in the factor(s) stimulating and maintaining normal and compensatory renal growth[10] and that the involvement of cellular membranes in the initiation of compensatory kidney growth is different from that in normal kidney growth.

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Possible involvement of calmodulin in apical constriction of neuroepithelial cells and elevation of neural folds in the chick[1]

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Summary. Chlorpromazine and trifluoperazine HCl, antipsychotic drugs known to bind to calmodulin, reversibly inhibited elevation of neural folds by interfering with the contractile activity of apical microfilament bundles in developing chick neuroepithelial cells.
Key words. Chick embryo; neurulation; microfilaments; calmodulin; phenothiazine antipsychotic drugs.

Schroeder[2] originally suggested that microfilaments in developing neuroepithelial cells are structurally and functionally comparable to muscle actin. This was later confirmed by the observations that 1) these microfilaments have the ability to bind heavy meromyosin to form characteristic arrowhead configurations[3] and 2) following indirect immunofluorescence staining with anti-actin antibodies, intense fluorescence is always found in apical regions of neuroepithelial cells where microfilaments are organized into prominent bundles[4,5]. It is well known that muscle contraction is regulated by local modulation of intracellular free calcium ion (Ca^{++}) levels. Thus a question arises as to whether or not Ca^{++} also serves as a regulator of microfilament contraction in the developing neuroepithelium in a manner similar to that of skeletal muscle. This view is supported, at least in part, by the finding that chemical agents (e.g., papaverine and ionophore A23187), which alter intracellular free Ca^{++} levels, disrupt elevation of neural folds through their action on the contractile activity of microfilaments[6-8]. Although troponin C, the skeletal muscle calcium-binding protein, has not yet been identified with certainty in non-muscle cells, studies have shown that Ca^{++} exerts control over a variety of cellular functions through a similar protein called calmodulin[9]. The recent demonstration that chlorpromazine (CPZ) and trifluoperazine HCl (TFP) bind specifically, in a calcium-dependent fashion, to calmodulin has raised the possibility that these antipsychotic drugs can be used to assess the contributory role of calmodulin in morphogenetic movements[10-13]. As a first part of our interest in this problem, we investigated the effects of CPZ and TFP on apical constriction of neuroepithelial cells and elevation of neural folds in the chick.

Materials and methods. Calmodulin antagonists (CPZ and TFP) were obtained from Smith Kline and French, Co. Fertile White Leghorn eggs were incubated at 37.5 °C to obtain embryos at stage 8 of development[14]. These embryos were chosen for investigation because the forming neural tube exhibits a gradual variation in the degree of openness along its length, providing an excellent opportunity to study the effects of chemical agents on closure of the neural tube[5]. Embryos were explanted using New's[15] technique and grown on medium (thin albumen) with or without a calmodulin antagonist. They were examined at intervals under a dissecting microscope to determine the degree of folding of the neuroepithelium. Unless otherwise indicated, several embryos were randomly selected from each treatment group after 6 h of incubation and processed for scanning and transmission electron microscopy (SEM and TEM)[5] to examine ultrastructural changes caused by the drugs.

Results and discussion. In a series of four experiments, 98 chick embryos were explanted at stage 8, cultured on thin albumen with or without a calmodulin antagonist, and examined at 2-h intervals (up to 6 h) under a dissecting microscope. At recovery, all (18) of the control embryos had advanced to stage 8+ or 9− of development. They had 5–6 somite pairs, and their neural folds in the future midbrain and anterior portion of the hindbrain already had made contact (fig. 1). In contrast, embryos responded to CPZ and TFP in a dose-related manner. Elevation of neural folds was inhibited in over 80% of the embryos by 40 μg/ml CPZ or 9 μg/ml TFP (fig. 2; table), whereas lower concentrations (e.g., 10 μg/ml CPZ and 3 μg/ml TFP) had no apparent effect on the morphogenesis of embryos. Higher concentrations (e.g., 80 μg/ml CPZ and 15 g/ml TFP) were deleterious and less than 10% of the embryos remained viable after 6 h

Effects of CPZ (40 μg/ml) and TFP (9 μg/ml) on elevation of neural folds in chick embryos explanted at stage 8 and cultured for 6 h

Appearance of neural folds in future midbrain region	No. and % (in parentheses) of embryos		
	Control	CPZ	TFP
Neural folds made contact (fig. 1)	18 (90.0)	2 (8.3)	0 (0.0)
Neural folds further elevated but showed no signs of contact	6 (10.1)	2 (8.3)	3 (12.5)
Neural folds appeared to be 'relaxed' (fig. 2)	0 (0.0)	20 (83.3)	21 (87.5)